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ABSTRACT

Iron deficiency anemia impairs cognitive performance, physical capacity, and thermoregulation. Recent evidence suggests that these functional impairments are also evident in subclinical nonanemic iron deficiency. Very little is known about the relevance of the latter to the health of blacks, who have been shown to have the highest prevalence of iron deficiency among all ethnic/racial groups in the United States, and other minorities. The Third Annual Nutrition Workshop was intended to fill this and other related important gaps. These conference proceedings include papers on the following topics: "Functional Significance of Iron Deficiency"; "Dietary Habits of Blacks and Other Ethnic Minorities in the U.S. with Special References to Iron Status"; "Iron Deficiency Diagnosis"; "Dietary Factors Influencing Bioavailability of Dietary Iron"; "Maternal Iron Deficiency and Pregnancy Outcome"; "Iron Deficiency: Pediatric Epidemiology"; "Iron Deficiency Coexisting with Sickel Cell Anemia"; "Iron Deficiency, Thyroid Function, and Thermoregulation"; "Immunity and Infection in Iron Deficiency"; "Iron Overload in Different Population Groups"; "Malnutrition, Work Performance and the Role of Iron"; "Iron in the Central Nervous System"; "Relationship of Brain Iron to Dopaminergic Neurotransmission and to the Genesis of Parkinson's Disease"; "Iron Deficiency: Long-Term Effects on Learning"; "Iron Deficiency and Childhood Lead Poisoning"; and "Iron Deficiency--Research Priorities." (IAH)

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Functional Significance of Iron Deficiency

Edited by
Cyril O. Enwonwu

Annual Nutrition Workshop Series
Volume III

CENTER FOR NUTRITION
Meharry Medical College

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Functional Significance of Iron Deficiency

Editor

Cyril O. Enwonwu, ScD, PhD, MDS
Director, Center for Nutrition,
Professor of Family and Preventive Medicine
Meharry Medical College
Nashville, Tennessee, USA

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PREFACE

Iron, the most abundant trace element in the human body, is critical to the functions of various redox enzymes and oxygen carrying proteins. On a global basis, iron deficiency anemia resulting from poor availability of the mineral from some diets or from chronic blood loss elicited by parasitic infestations, is the most prevalent nutritional disorder in the human. It is widespread in the developing world. In industrialized countries, the prevalence rates are as high as 10-20 percent, with women of child-bearing age and the socioeconomically disadvantaged constituting the most vulnerable groups.

Iron deficiency anemia impairs cognitive performance, physical capacity and thermoregulation, among other biological functions. More disturbing are relatively recent pieces of evidence suggesting that these functional impairments are also evident in subclinical nonanemic iron deficiency. Very little is known about the relevance of the latter to the health of blacks and other minorities who constitute a disproportionately large fraction of the impoverished in this country, and who have been shown to have the highest prevalence of iron deficiency among all ethnic/racial groups in the U.S. The Third Annual Nutrition Workshop was intended to fill this and other related important gaps.

The workshop was held at the Kresge Learning Resource Center, Meharry Medical College on October 25-27, 1989. The keynote address for the three-day meeting was delivered by Nevin S. Scrimshaw, M.D., Ph.D, who discussed the several overlapping stages of iron status ranging from iron excess to iron deficiency anemia, pointing out that much earlier biochemical changes and not the reduction in circulating hemoglobin level, constitute the primary factors responsible for the alterations in muscle and central nervous system functions in iron deficiency. Other important topics discussed at the workshop included dietary habits of blacks and other ethnic minorities in the U.S. with special reference to iron status, diagnostic criteria for iron deficiency, thyroid function and thermoregulation in iron deficiency, role of iron status on work performance, relationship of iron deficiency to dopamine receptors in the brain and to the genesis of Parkinson's Disease, and the long term effects of iron deficiency on learning. Attempts were also made to identify areas requiring urgent research attention.

Financial support for this workshop was provided by Kraft/General Foods, Inc., the U.S. National Institutes of Health, Best Foods, Ross Laboratories, and the Philip Morris Companies, Inc. Meharry Medical College is profoundly grateful to these organizations for the generous support.

Cyril O. Enwonwu

WELCOME ADDRESS

David Satcher, M.D., Ph.D.
President, Meharry Medical College

Thank you very much, Dr. Carter. I am delighted to have the opportunity on behalf of Meharry Medical College, to welcome you here to the Third Annual Workshop on nutrition organized by the Center for Nutrition of this institution. We are delighted to have such a distinguished group of people and faculty gathered here for this conference. The nutrition center means a lot to our institution. Dr. Enwonwu has done a very good job of developing the center and impacting upon the teaching programs of our institution. As you probably know, we have four schools; School of Medicine, School of Dentistry, School of Graduate Studies and School of Allied Health. It is not easy to change curriculum —you know that. Somebody said that changing the curriculum of medical schools is like moving a cemetery with a lot of dead bodies. It is tough. Dr. Enwonwu has had to do that and he's been able to get the cooperation of faculty, deans and others. We feel very good about the nutrition center, what it has accomplished, and about having you here to contribute to the dynamics of the development and sharing of information as it relates to nutrition, especially in the important area of iron deficiency anemia. We hope that your stay here will be a pleasant one.

Meharry is an historic institution. We just held our one hundred and fourteenth convocation. On that occasion, Dr. Louis Sullivan, U. S. Secretary of Health,, was our keynote speaker. We also initiated the Institute on Health Care for the Poor and the Underserved at Meharry. This institute will deal with policy issues, and will be a national clearinghouse of information. It will sponsor an annual conference, and will also, beginning in the winter, have a journal on health care for the poor and the underserved. I hope that in time, many of you will contribute to the journal. The journal needs to be successful. There are lots of issues, financial and otherwise related to health care for the poor and the underserved in this country that need to be aired and dealt with by scholars. So we hope that the journal will get the kind of support throughout the country that it needs at this time in our history. Again, I'd like to say that I welcome you on behalf of the entire Meharry Medical College family and we are delighted to have you with us.

Thank you.

Contents **Page**

Welcome Address - David Satcher
President
Meharry Medical College

Keynote Address - Functional Significance of Iron Deficiency 1
Nevin S. Scrimshaw

Dietary Habits of Blacks and other Ethnic Minorities
in the U.S. with Special References to Iron Status 15
Ann C. Looker, Catherine M. Loria,
Margaret A. McDowell and Clifford L. Johnson

Iron Deficiency Diagnosis 25
Francisco Chew

Dietary Factors Influencing Bioavailability of Dietary Iron 33
Eugene R. Morris

Maternal Iron Deficiency and Pregnancy Outcome 45
Bonnie Worthington-Roberts

Iron Deficiency: Pediatric Epidemiology 57
Allan A. Johnson

Iron Deficiency Coexisting with Sickel Cell Anemia 67
Clyton L. Natta

Iron Deficiency, Thyroid Function, and Thermoregulation 71
John Beard

Immunity and Infection in Iron Deficiency 81
Gearld T. Keusch

Iron Overload in Different Population Groups 93
Victor R. Gordeuk

Malnutrition, Work Performance and the Role of Iron 103
G.B. Spurr

Iron in the Central Nervous System 117
Theodore L. Sourkes

Relationship of Brain Iron to Dopaminergic
Neurotranmission and to the Genesis of Parkinson's Disease 129
Moussa B.H. Youdim

Iron Deficiency: Long-Term Effects on Learning 139
Harold H. Sandstead

Iron Deficiency and Childhood Lead Poisoning 147
Ray Yip

Iron Deficiency—Research Priorities 153
Ray Yip

Closing Remarks 160
George Hill
Director of Biomedical Sciences and Associate Dean

KEYNOTE ADDRESS

FUNCTIONAL SIGNIFICANCE OF IRON DEFICIENCY

Nevin S. Scrimshaw, Institute Professor Emeritus
Massachusetts Institute of Technology, Cambridge
and
Director, Food, Nutrition and Development Programme
United Nations University, Tokyo

Introduction

Iron deficiency is the most widespread nutritional problem in the world today and, unlike the classical deficiency diseases, also still affects substantial numbers of persons in the United States and other industrialized countries. A recent report from India gives an anemia prevalence of 60-80% for pregnant women in different regions of India (1). If iron deficiency rather than anemia is the criterion, it has been suggested that one-third of premenopausal women in the United States fall into this category, with minority groups more heavily affected.

Using data from NHANES II for 1976-80, the Life Sciences Research Office (2) reported high frequencies of impaired iron status in U.S. preschool children and premenopausal women, particularly among those whose incomes are below the poverty level. The report documents an overall 7.2 per cent prevalence of actual anemia in women 15 to 44 years of age, but with the highest burden in minority and poverty groups. For example, the 1976-80 NHANES II figure for the percentage of persons with two or three abnormal iron status indicators was 11.7% for white and 31.3% for black adolescents 15 to 19 years of age.

There are several overlapping stages of iron status ranging from iron excess to iron deficiency anemia. Anemia is generally diagnosed on the basis of the WHO standards for various age and sex groups (3). For determining iron status the criteria shown in Figure 1 have been proposed for adults, with small adjustments for younger age groups (2). In the first stage of iron deficiency, plasma transferrin is increased and ferritin markedly decreased. Erythropoiesis is affected when, in addition, plasma iron and transferrin saturation are decreased and iron protoporphyrin elevated.

In iron deficiency anemia these values are further altered, and the erythrocytes are seen to be microcytic and hypochromic. Intestinal iron absorption increases progressively with iron depletion. It is now clear that earlier biochemical changes and not the reduction in circulating hemoglobin are the primary factors responsible for the alterations in muscle and central nervous system function in anemic subjects. With iron overload, plasma iron and ferritin levels as well as iron deposits in various tissues are greatly increased and transferrin iron-binding capacity is elevated.

While approximately 73% of the body's iron is normally incorporated into hemoglobin and 12% in the storage complexes ferritin and hemosiderin, a very important 15% is incorporated into a variety of other iron-containing compounds, some of them enzymes of vital importance. The heme compounds include myoglobin, cytochromes, catalases and peroxidases. The non-heme compounds include NADH and succinic dehydrogenases; xanthine, aldehyde and alphaglycerophosphate oxidases; phenylalanine hydroxylase; and ribonucleotide reductase. Alphaglycerophosphate oxidase, for example, shuttles electrons across the mitochondrial membrane. Also important are the iron-dependent enzymes: lipid peroxidase, proline and lysine hydroxylase and a number of others.

While there can still be considerable discussion of the best cutoff points for maximum sensitivity and specificity of diagnosis in various circumstances, the important issue is their application to the identification of vulnerable groups and the functional significance of these categories. Although the papers to follow in this symposium will cover each of the topics in more detail, I would like to give an overview of what is known of the impact of these stages of iron status on various functions.

Human reproduction

In normal men the combined daily loss of iron through the urine, skin and feces is only about 1 mg. The iron requirements of women under various circumstances are analyzed in detail in an excellent INACG publication (4). Because of their smaller body size, losses in women from these sources is less, perhaps 0.7-0.8 mg daily, but median menstrual losses averaged over a month add the equivalent of another 0.4 - 0.5 daily. However, these menstrual losses are very asymmetrically distributed, with 10% of normal women experiencing losses three times the median. In some women the use of conventional intrauterine devices for contraception leads to increased vaginal blood loss. Even without other adverse factors these women are at obvious risk. While menstruation temporarily ceases during pregnancy, the overall iron cost to the mother is greater, around 5 mg. daily, during the second and third trimesters. To this must be added the iron lost in blood at delivery and in lochia later in the puerperium, around 150 mg; but once again, there is great individual variation.

For underprivileged populations, poor iron availability and often pathological iron losses associated with parasites greatly increase the prevalence of iron deficiency in women who, already in borderline status, are the most seriously affected. Under these circumstances the physical demands on women for procuring food and other economic activities as well as food preparation and family and home maintenance are great, and iron deficiency reduces their capacity to meet these demands.

In addition to the consequences of iron deficiency affecting all persons, to be discussed later, there are additional adverse consequences of iron deficiency in childbearing women. Maternal mortality, prenatal and perinatal infant loss, and prematurity are significantly increased. If the mother is iron deficient during pregnancy, her infant is born with poor iron reserves and is at greater risk of morbidity and mortality during infancy (4). Low-birth-weight infants exhaust their iron stores at an earlier age and require more iron than supplied by breast milk at an earlier age than infants of normal birthweight (5, 6).

Child Growth

In three separate studies in Indonesia, iron supplementation resulted in significantly increased growth of children (7, 8) compared with that of children receiving placebo. Since no other study using iron supplementation appears to have included growth measurement, there is a need to confirm this finding in other populations. If confirmed, the result could be due either to a direct effect of iron itself or to increased food intake because appetite is enhanced in persons whose iron deficiency is reversed.

Physical capacity

Well before studies with human subjects, the effect of iron deficiency on physical performance was explored in experimental animals. Edgerton et al. (9) showed a linear relationship between hemoglobin and change in spontaneous activity in rats relative to control rats (Figure 2). A similar relationship was found in rats for running time to exhaustion. Figure 3 from Finch et al. (10) shows the poor treadmill performance of rats on an iron deficient diet for 3 and 4 weeks compared with groups receiving adequate iron, and a progressive improvement in iron deficient rats with supplementation. Other workers (11,12,13) report similar results.

These observations were replicated later in concurrent studies of human subjects in Guatemala and Indonesia. Figure 4, from Viteri et al. (14), shows a linear correspondence between hemoglobin status and Harvard step test (HST) results. In subjects this performance improved with iron supplementation. In Indonesia Basta et al. (15) found similar differences in HST scores between anemic and non anemic road construction workers and rubber tappers who had improved performance when they were given iron for 30 days, before significant improvement in hemoglobin status.

Among male tea pickers in Sri Lanka the results were similar (16). In both Sri Lanka and Kenya treadmill was proportional to plasma hemoglobin. Spurr et al. (17) also found linear correlations between hemoglobin status, aerobic power and other measures of physical capacity in Colombian laborers.

Work performance and productivity

The question remains as to whether these observed differences in physical capacity affect productivity. Basta et al. (15) subsequently showed a strong correlation between hemoglobin status, HST performance and the take-home pay of Indonesian rubber tappers (Figure 5). Tappers given iron supplementation for 60 days increased their take-home pay by more than 30%. Among the weeders who were not paid on an incentive basis less area was weeded by those who were anemic, and their output also increased with supplementation.

In a subsequent study in Indonesia by Husaini et al. (18), the mean amount of tea leaves collected per hour was significantly less for anemic women; work output increased by 24% after four months of iron supplementation. Similar results were obtained in a larger follow-up study sponsored by UNU on four other tea plantations in the same area. Figure 6 shows that the daily productivity of the anemic male tea pickers in Sri Lanka increased more than 20% after iron supplementation for 1 month (19,20). An increase in agricultural productivity of Indian women (21) given iron supplementation has been reported. Productivity also increased with the iron supplementation of anemic male agricultural workers in Colombia (17).

Morbidity from infection

In experimental animals, iron deficiency has been associated with the severity of hookworm infection in rats (22), cats and dogs (23), and with increased morbidity and mortality in rats experimentally infected with salmonella (24).

Poorly nourished infants in London were reported in 1928 to have less bronchitis and gastroenteritis when they received iron supplementation (25). However, considering the widespread occurrence of iron deficiency, subsequent observations on the effect of iron deficiency on morbidity from infections are relatively meager. The first modern controlled observation that I could identify came nearly 40 years later and demonstrated that infants receiving a proprietary formula with vitamins and iron had approximately half the respiratory infections of infants given the same formula unsupplemented (26).

Iron deficiency in Alaskan native children has been reported to be associated with increased diarrheal and respiratory disease (27), and meningitis was observed to be fatal only in anemic children (28). The study of Basta et al (15) in Indonesia, mentioned earlier, found that the greater morbidity from infection among the anemic rubber tappers decreased after iron supplementation. In both the Egyptian (29) and Indonesian (18) field studies, a decrease in diarrheal and respiratory infections was observed in the groups receiving iron supplementation.

There is evidence from Guatemala that vitamin A deficiency exacerbates iron deficiency (30), and from both Guatemala and Indonesia (31), that supplementation of poorly nourished child populations with vitamin A results in improved iron nutriture. The implications of this interaction have not been explored. However, the administration

of vitamin A in periodic massive doses in Indonesia (32), and similar programs in progress in a number of other countries, have shown significant reductions in morbidity and mortality from infections (33). Some or all of this may be mediated through the effect of vitamin A on their iron status.

Deficiency and immunity

Information on mechanisms whereby iron deficiency results in increased morbidity is also relatively limited, but a number of antimicrobial systems (within the neutrophil) are adversely affected by iron deficiency. In the study previously referred to, of experimental salmonellosis in rats, by Baggs and Miller (24), both viable intracellular and extracellular bacteria were observed in greater numbers in the macrophages and in the intestinal wall of the iron-deficient rats compared with iron replete animals. This is paralleled by the concentrations in the intestinal wall of the iron-containing enzyme myeloperoxidase that mediates the iodination of proteins and the formation of hydrogen peroxide to kill microorganisms within the cell (Figure 7).

Superoxide produced by the action of the enzyme superoxide dismutase is also involved in the respiratory burst that is part of the mechanism for killing organisms ingested by leukocytes (34). Chandra et al. (35) have demonstrated in Indian children that the mitogenic response of the lymphocytes, the capacity for respiratory bursts necessary for killing ingested organisms, and the number of intracellular bacteria in lymphocytes exposed in culture are related to transferrin saturation (Figure 7). The relationship between hemoglobin status and in vitro bactericidal activity is shown more clearly in Figure 8 (35).

There is extensive evidence from India (36, 37, 38) for a direct relationship between iron status and plasma T- lymphocyte concentrations. Impaired delayed cutaneous hypersensitivity to a number of ubiquitous antigens has also been described in iron-deficient children in India (36) and Kenya. Additional evidence for the effects of iron deficiency on immunity has been reviewed by Keusch (39).

Cognitive performance and behavior

Recently, more attention has been paid to the relationship between iron status and cognitive performance than to any other functional consequence of iron deficiency. While there were a number of relevant earlier studies in experimental animals (40-47), in 1973, Webb and Oski (48) reported improvement in the cognitive performance of iron-supplemented adolescents, but since there was no control group a practice effect was suspected. In 1978, Oski and Honig showed an improvement in the Bailey scores of anemic infants given iron supplementation compared to those achieved by a placebo group (49).

I believe that credit for the first demonstration of an adverse effect of iron deficiency even without anemia belongs to Pollitt and coworkers (50, 51). They found that, on a battery of cognitive and behavioral tests, mildly iron deficient 3-6 year old children in Cambridge, Massachusetts had lower scores than those with normal iron status, but the low scores returned to normal after children were given 11-12 weeks of supplementary oral iron. Pollitt then repeated these studies with preschool children in Egypt and Indonesia (52). Figure 9, based on the Cambridge data, typifies the results in Cambridge and Indonesia, but with the moderate to severe anemia of children in Guatemala and Egypt, there was no significant cognitive test improvement in anemic children within the two to three month period of supplementation, even though their hematological deficit was corrected.

Concurrently with these studies, Lozoff in Guatemala (53) and subsequently in Costa Rica (54), Stekel (55) and later Walter (56, 57, 58) in Chile, tested anemic and control infants using the Bailey scale. Table 1 typifies the findings of impaired performance in the anemic groups in these studies. However, except in the Stekel (55) and Oski and

Honig studies (49), most of the children failed to respond to even three months of supplementation, raising the alarming possibility that the effects of anemia are lasting.

Two studies by Soemantri et al., in Indonesian school children (7, 8) not only documented poor performance by those initially iron-deficient, but they also observed substantial recovery after 12 weeks of iron supplementation. The results in four studies of school-age children in India were similar (59). However, a double-blind study of 1358 children 9 - 11 years old by Pollitt et al. (60) in Thailand found a positive association between iron status and performance on the Raven Progressive Matrices used to measure IQ, a Thai language and a math test. It is noteworthy that children who were iron-depleted without anemia obtained significantly lower scores in Thai language than did iron-replete children. While the IQ and math scores were also lower in the iron-depleted, the difference was not statistically significant. However, no significant improvement was observed following 100 mg of iron as ferrous sulfate per day for 14 weeks. The overall results of these various studies suggest that iron deficiency is educationally disadvantageous regardless of ethnicity and physical and social environment.

It is evident that key iron-containing compounds in the brain respond to iron deficiency even when the deficiency is not sufficiently severe to affect hematopoiesis. Recent work from Israel with iron-deficient rats (61, 62) suggests that selective diminution of central dopamine neurotransmitters may be a key mechanism. It is postulated that interference with iron metabolism at an early age could result in irreversible damage to developing dopamine neurons, with consequences evident in later life.

Temperature regulation

There are a number of studies reporting that iron- deficient rats exposed to low environmental temperatures are unable to maintain their body temperatures (63, 64, 65). There is an associated increase in plasma norepinephrine and a retarded conversion of the thyroid metabolite T4 to T3. Figure 10 is an example of the findings in these animal studies. These findings have been replicated with volunteer human subjects in Caracas, Venezuela, by exposing them for one hour successively to water baths of 36°C and 28°C (M. Layrisse, personal communication).

Overload and infection

It is important for this workshop to discuss the consequences of excessive dietary iron as well as iron deficiency for two reasons. First, there are circumstances in which the administration of parenteral or large oral doses of iron can lead to overwhelming infections. Second, failure to understand the special circumstances under which this occurs can interfere with needed programs of iron supplementation and fortification for populations at risk of iron deficiency.

It is not only the host that needs iron for the biochemical functions mentioned in the introduction, but also the infectious agent (66). Without it, replication is inhibited. In fact, withholding iron from the infectious agent appears to be an important mechanism of resistance to infections (67, 68). Conalbumin and lactoferrin have stronger iron binding properties than most bacterial siderophores and are normally highly unsaturated and function as iron-withholding rather than iron-transport agents.

Lactoferrin, known to be released upon degranulation of leukocytes in aseptic areas, is a major component of human milk and resists proteolytic destruction in the gastrointestinal tract. It is not difficult to demonstrate in vitro the protective effect of lactoferrin (69). In the iron- deficient host with reduced cell mediated immunity and leukocyte function, lack of available iron for agent replication is protective. Baggs and Miller (24) found that in rats exposed to a standard dose of Salmonella, a diet lacking in iron is almost as protective as one meeting iron needs.

Murray and Murray have described the

exacerbation of malaria with a high case fatality rate in Somalia refugees given therapeutic doses of iron (70, 71). Parenteral iron given to children with kwashiorkor has also been associated with mortality from overwhelming infection (72, 73). However, in our supplementation studies in Egypt (29) and Indonesia (31), morbidity from diarrheal and respiratory disease decreased promptly with iron supplementation. The iron supplement promoted recovery of immune function without providing enough iron to increase the severity of existing or subsequent infections.

Iron Status and cancer

The final topic to be considered is the possibility of a relationship between high iron intakes and the risk of some kinds of cancer (74-77). These reports have presented evidence that increased body iron stores, as judged from serum ferritin levels, are associated with an increased cancer risk. Although these studies are preliminary and inconclusive, they provide an additional reason for avoiding iron excess as well as iron deficiency The mechanisms involved may be comparable to those seen in the effect of iron overload on infections, i.e., adverse effects on the immune system and a reduction in capacity to lower rapidly replicating cells.

Summary

In summary, iron deficiency has been shown to affect adversely the physical capacity and work performance of adolescents and adults, the cognitive performance and behavior of infants, preschool and school children, immune status and morbidity from infections of all age groups, and the maintenance of body temperature in adults exposed to a cool environment. In general, these effects are corrected by iron supplementation, but in the case of children with moderate to severe iron deficiency anemia, the effects on cognition may not be reversible.

In addition to a genetic defect resulting in excessive iron absorption and overload leading to serious liver pathology, excessive dietary iron may produce the same results in exceptional circumstances. In children and adults who are immunologically compromised by severe malnutrition and heavily exposed to infection, the administration of parenteral iron or a large dose of oral iron may result in overwhelming infection. Excessive iron intakes may also favor some forms of cancer. However, the amount of iron provided in programs of fortification of staple foods and in routine prenatal iron supplementation has been repeatedly demonstrated to improve reproductive performance, enhance resistance to infections, and increase work capacity and productivity without observable risk.

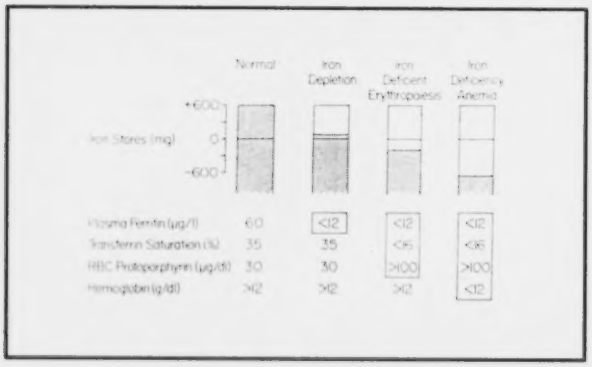

Figure 1. Measurements of iron status in relationship to body iron stores (mg). Negative iron stores indicate the amount of iron that must be replaced in circulating red cells before iron reserves can re-accumulate. Ref. 3.

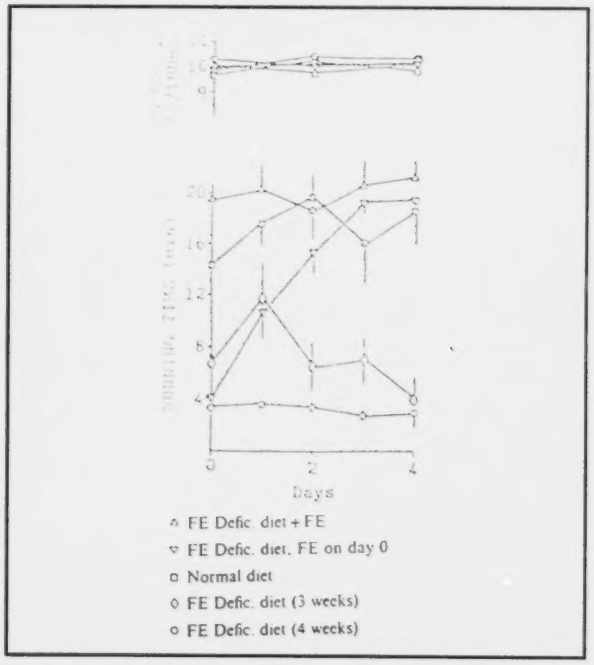

Figure 3. Work performance at 10g/100ml hemoglobin by normal and iron-deficient animals (Finch *et al.*, 1976 [7]). Ref. 10.

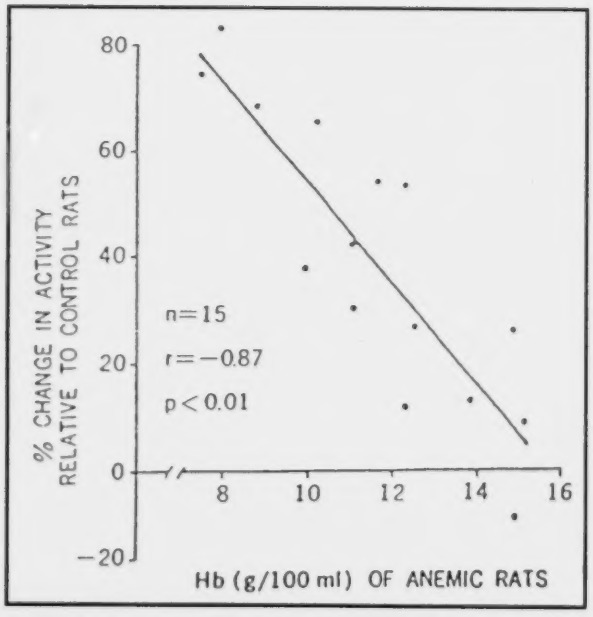

Figure 2. The relationship between Hb and percent differences in voluntary activity between anemic and control groups is shown. Percent change in activity=-9.56 (Hb)+149.59. Ref. 9.

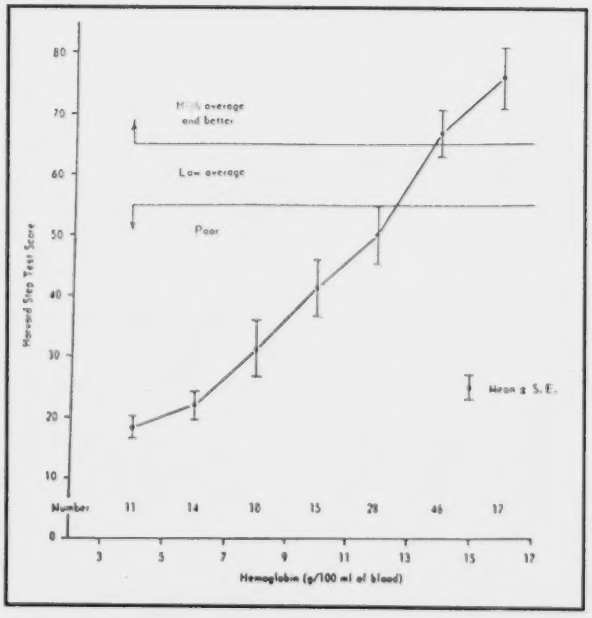

Figure 4. Physical fitness in relation to hemoglobin concentration in Guatemalan agricultural workers. Ref. 14.

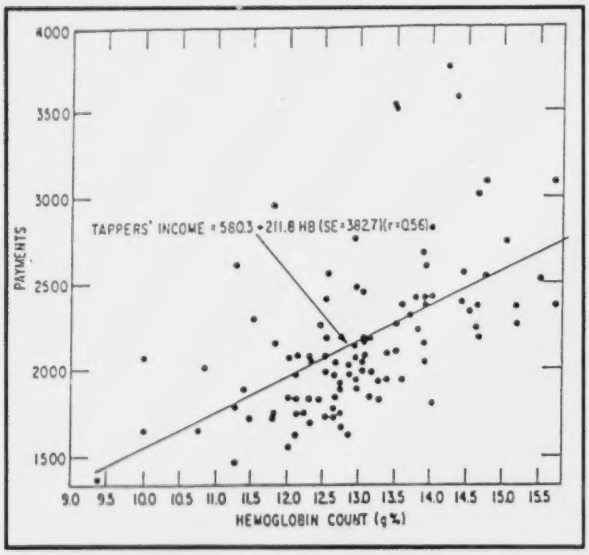

Figure 5. Hemoglobin versus Tappers' income (preintervention program). Ref. 15.

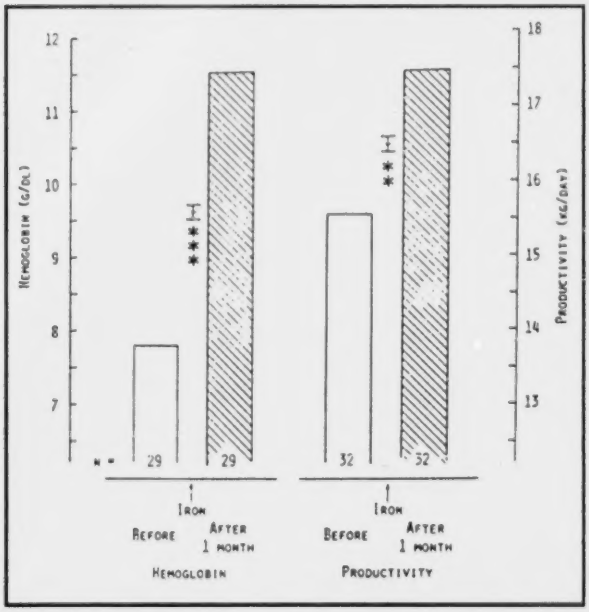

Figure 6. Changes in Hb and daily productivity (tea plucked) in response to oral iron treatment in subjects whose initial Hb levels were less than 9.0 g/dl. $^{**}p<0.01$, $^{***}p<0.001$, and vertical bars (mean $\pm$ SEMD) were obtained from paired *t*-tests between the values before and 1 month after iron treatment. *N* represents the number of subjects. Ref. 19-20.

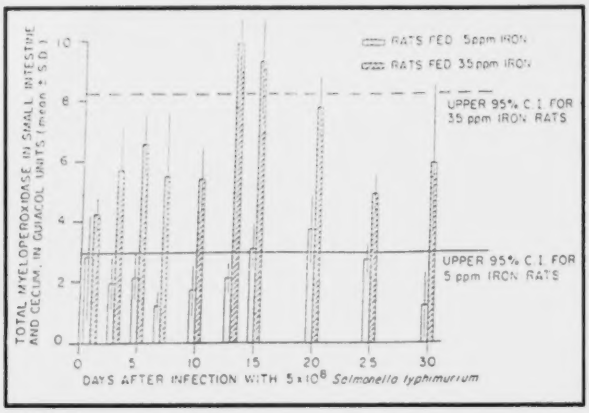
RATS FED 5ppm IRON
RATS FED 35ppm IRON
UPPER 95% C.I. FOR 35 ppm IRON RATS
UPPER 95% C.I. FOR 5 ppm IRON RATS
TOTAL MYELOPEROXIDASE IN SMALL INTESTINE AND CECUM, IN GUAIACOL UNITS (mean ± S.D.)
DAYS AFTER INFECTION WITH 5x10⁸ Salmonella typhimurium

Figure 7. Ref. 24.

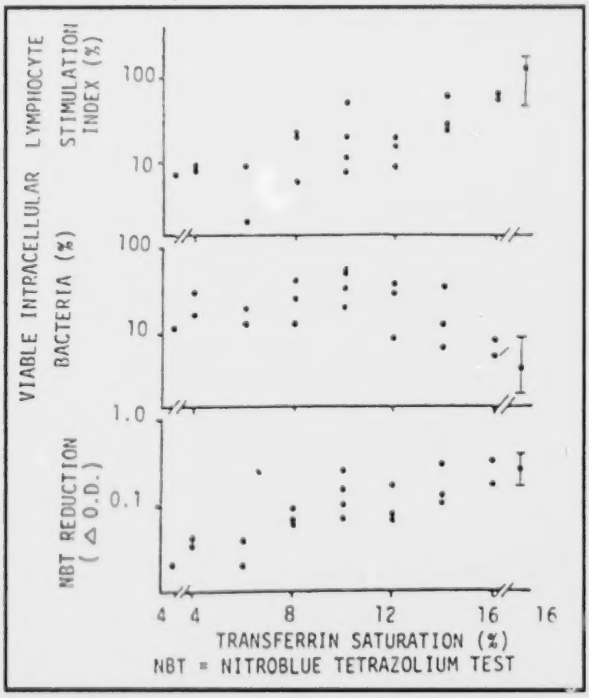
VIABLE INTRACELLULAR BACTERIA (%)
LYMPHOCYTE STIMULATION INDEX (%)
NBT REDUCTION (Δ O.D.)
TRANSFERRIN SATURATION (%)
NBT = NITROBLUE TETRAZOLIUM TEST

Figure 8. Correlation between depressed *in vitro* response and transferrin unsaturation in children (32). NBT, nitroblue tetrazolium test. Ref. 35.

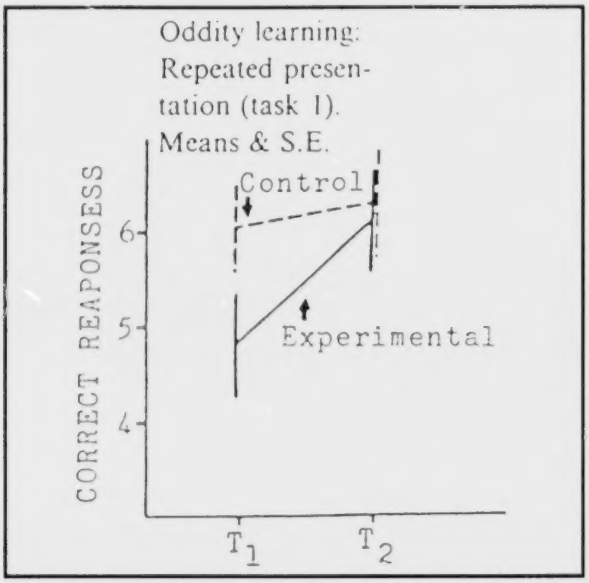

Figure 9. Performance of control and experiental groups of children on oddity learning tasks before (T_1) and after (T_2) administration of oral iron. Ref. 50.

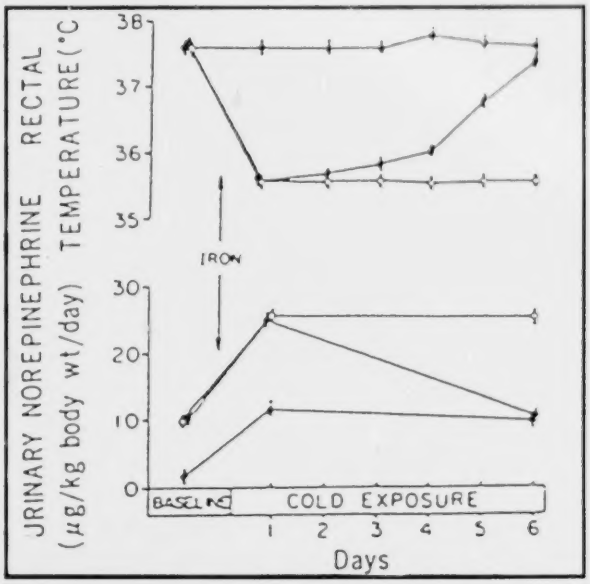

Figure 10. Effects of iron treatment on temperature regulation and urinary catecholamines in iron-deficient and control rats (Dillman *et al.*, 1982 [49]). Iron deficient rats, ○; iron deficient rats treated with iron. ○: control rats. ○.

Table 1.

Behavior pattern	Number of infants		Exact probability
	Anemic	Non-anemic	
Withdrawn or hesitant	6	2	0.05
Fearful	7	2	0.02
Tense	7	1	0.01
Unreactive to usual stimuli	7	3	0.05
Decreased bodily activity	7	4	0.07
Lack of persistence	8	5	0.10

Ref. 52

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DIETARY HABITS OF BLACKS AND OTHER ETHNIC MINORITIES IN THE U.S. WITH SPECIAL REFERENCES TO IRON STATUS

Anne C Looker *, Catherine M Loria, Margaret A McDowell, and Clifford L Johnson.

Division of Health Examination Statistics,
National Center for Health Statistics,
Hyattsville, MD 20782

Introduction

Ethnicity is one of many factors known to influence dietary habits [1,2]. Most of the studies relating diet to ethnicity performed to date have focused on dietary habits in general rather than specifically on nutrients and foods relevant to iron status. Furthermore, it has not been possible to examine dietary habits of minorities other than blacks with databases such as the National Health and Nutrition Examination Surveys (NHANES) or the Nationwide Food Consumption Survey (NFCS) until recently, because the samples used in these surveys did not contain adequate numbers of other minority groups to permit analyses.

A complete examination of dietary habits also needs to include vitamin-mineral supplements (VMS), given their widespread use in the population [3,4]. Differences in overall VMS use among minorities have been noted [3,4], but more detailed information on the nutrient composition of the VMS has not been available to permit an examination of nutrient intake relevant to iron status.

The objective of this study was to use data from nationally representative samples to provide an overview of dietary habits, including VMS use, relevant to iron status among minorities in the U.S. Specifically, intakes of nutrients and food groups relevant to iron status were compared between non-Hispanic blacks (NHB), non-Hispanic whites (NHW), Mexican Americans (MA), Cubans, and Puerto Ricans (PR) in selected age groups using data from the second National Health and Nutrition Examination Survey (NHANES II) and the Hispanic Health and Nutrition Examination Survey (HHANES). In addition, VMS use was compared between NHB, NHW, and Hispanics in selected age groups using data from the 1986 National Health Interview Survey (NHIS).

Methods/Sample

Results for nutrient and food group intake presented for NHB and NHW are from data collected in the NHANES II, while those presented for MA, Cubans, and PR are from data collected in HHANES. Both surveys were conducted by the National Center for Health Statistics. NHANES II, conducted from 1976-80, was a national probability sample of the non-institutionalized US population aged 6 months—74 years [5]. HHANES, conducted from 1982-84, was a probability survey of three Hispanic subgroups aged 6 months—74 years in selected areas: MA from five southwestern states (Arizona, California, Colorado, New Mexico, and Texas); Cubans from Dade County, Florida; and PR from the New York City metropolitan area [6]. The MA, Cubans, and PR living in these three areas represented approximately 84 percent, 57 percent, and 59 percent of the 1980 MA, Cuban, and PR populations in the US, respectively. Because certain locations that contained too few Hispanics within the three areas were excluded at the outset of the survey, not all Hispanics residing in these areas

*Participant

were eligible to be sampled in HHANES. Thus, 97 percent of the 1980 MA population in the five southwestern states, 96 percent of the Cuban population in Dade County, and 90 percent of the PR population in the New York City metropolitan area were eligible for HHANES.

The analytic samples from NHANES II and HHANES for this study were restricted to three age groups: 2-6 years, 18-44 years, and 65-74 years. These groups were chosen to allow examination of dietary habits at different ages and to be consistent with the analytic sample from NHIS used to examine VMS use. Pregnant women were excluded from the analytic sample, since food habits practiced during pregnancy may not represent habits practiced at other times. Finally, persons who lack values for nutrient or food group intake were excluded from the analysis of that particular variable.

To form the analytic sample of NHB and NHW from NHANES II, we excluded persons whose self-reported family ancestry or national origin was one of the following: a) Chicano; b) Mexicans; c) Mexicano; d) Mexican American; e) other Spanish; f) countries of Central or South America; g) Puerto Rican; or h) Cuban. From the 10,815 non-Hispanic persons aged 2-6, 18-44, or 65-74 years examined in NHANES II, 10,712 remained in the analytic sample after all exclusions had been made. The analytic sample of non- Hispanics consisted of 1,540 NHB and 9,172 NHW.

It was also necessary to use self-reported family ancestry or origin to restrict the analytic sample from HHANES to MA, Cuban, or PR, because, due to sampling rules that allowed all members of a family with at least one Hispanic member to be eligible, some non-Hispanic persons were included in HHANES. There were 5,450 Hispanic persons aged 2-6, 18-44, or 65-74 years examined in HHANES, of which 5,404 remained in the analytic sample after performing the exclusions. The analytic sample of Hispanics consisted of 3608 MA, 581 Cubans, and 1,215 PR. The sample sizes in each age, sex, and racial or ethnic group are shown in Table 1. The age, sex, and poverty status distributions of the sample before and after making the exclusions were similar, which suggested that no overt biases were introduced by the deletions.

Results for VMS use by NHB, NHW, and Hispanics are from data collected in the 1986 NHIS. The NHIS is a continuing cross- sectional survey of the civilian, non-institutionalized US population that is conducted via household interviews [3]. In 1986, 23,838 households were interviewed in the NHIS, which resulted in a sample of 62, 052 interviewed persons. The noninterview rate was 3.5 percent of all eligible households.

The items on VMS use were included in the NHIS during the six month period from January--July, 1986. The VMS subsample consisted of one randomly selected adult aged 18 years or older and one randomly selected child aged 2-6 years, if available, from each interviewed family. The final interviewed VMS subsample consisted of 13,652 persons: 11,775 adults and 1,877 children from 11,879 households. The overall response rate for the VMS subsample was 93.1 percent.

The analytic sample from the 1986 NHIS for this study was restricted to persons aged 2-6 years, 18-44 years, and 65+ years to be consistent with the analytic sample formed from NHANES II and HHANES as well as with previous analyses of the NHIS VMS use data [3]. Pregnant or lactating women and persons whose VMS use in the past 2 weeks was unknown were excluded from the sample.

We used self-reported Hispanic origin to form the analytic sample of NHB, Hispanics, and NHW from NHIS. Individuals were classified as Hispanic if they reported their national origin as one of the following: a) multiple Hispanic; b) Puerto Rican; c) Cuban; d) Mexican-Mexicano; e) Mexican American; f) Chicano; g) other Latin American; h) other Spanish; or i) Spanish, type unknown. The remaining individuals were classified as non-Hispanic. From the 10,866 persons aged 2-6, 18-44, or 65+ years interviewed in the VMS subsample in the 1986 NHIS, 10,359 remained in the analytic sample after all exclu-

Table 1. Sample sizes by age, sex, and racial or ethnic group in analytic samples

Survey Racial or ethnic group	Male 2-6 yr	Male 18-44 yr	Male 65-74 yr*	Female 2-6 yr	Female 18-44 yr	Female 65-74 yr*
A. Nutrient and food group intake analytic sample						
NHANES II						
Non-hispanic black	292	300	127	273	352	150
Non-hispanic white	1206	2133	1012	1133	2233	1210
HHANES						
Mexican American	586	1039	83	543	1238	119
Cuban	44	171	41	39	222	64
Puerto Rican	180	295	25	167	494	54
B. Vitamin-mineral supplement use analytic sample						
NHIS, 1986						
Non-hispanic black	169	346	111	182	580	160
Non-hispanic white	634	2285	663	620	2667	1231
Hispanic	107	215	26	84	247	30

*Age category from 1986 NHIS was 65+ years.

sions had been made. The analytic sample consisted of 1,548 NHB, 709 Hispanics, and 8,102 NHW. Sample sizes by age, sex, and ethnic group are shown in Table 1. Hispanics in the analytic sample from NHIS were not divided into the major Hispanic subgroups in order to preserve an adequate sample size when examining use of iron- and vitamin C-containing VMS. Comparison of selected characteristics of the sample before and after making the exclusions suggested that no overt biases were created by the deletions.

Variables

Two methods were used to measure dietary intake in NHANES II and HHANES: a) a single 24-hour recall, which provided estimates of energy and 17 nutrients; and b) a food frequency, which provided estimates of usual weekly intake of 18 food groups in NHANES II and of 57 foods or food groups in HHANES. In this study, we compared dietary intakes of energy, iron, and vitamin C from the 24 hour recall between NHB and NHW. The 24-recall data from HHANES were not available at the time of this analysis, so comparison of nutrient intakes was not possible for MA, Cubans, or PR. However, food frequency data were available from both surveys, so comparison of these data was possible among the five population groups. We examined intake of nine food groups that could influence iron status by providing dietary iron or factors that enhance iron absorption, such as vitamin C, meat, poultry, or fish [7,8]. The food groups were meat, organ meats, poultry, fish, mixed dishes that were high in protein, cereal, grain products, fruits and vegetables, and legumes. The foods included in each group have been described previously [5,6]

VMS users were defined as individuals who reported taking at least one nonprescription vitamin, mineral, or fluoride product during a two-week reference period before the interview. Persons taking only prescription VMS were not classified as supplement users in this study, in order to be consistent with previous analyses of these data [3]. The nutrient composition of the VMS was obtained directly from the product container by the NHIS interviewer. When no container was available, persons were asked to report the name and manufacturer of the product(s) taken. Descriptive characteristics of the respondents used in this study were age, sex, and poverty income ratio (PIR). The PIR is calculated by dividing the total household income by the poverty threshold for a household of that size. Persons whose family PIR was <1.0 were considered to be poor in this study, while those whose family PIR was >1.0 were considered nonpoor.

Data Analysis

Data were analyzed separately by sex to account for differences in dietary intakes between the sexes. To account for the complex design of both surveys, sample weights were used when calculating point estimates (eg, means, prevalences). The use of sample weights provides point estimates that represent the targeted US subpopulation at the midpoint of the respective survey. Variances were calculated by procedures that account for the complex survey design and for ratio adjustments used to produce sample weights. The effect of the complex sample design on variance estimates is reflected by the design effect, which is the ratio of the variance of a statistic from a complex sample to the variance of the same statistic from a simple random sample of the same size [9]. For NHIS data, variance estimates were cal-

culated using a Taylor series linearization method, which uses subgroup-specific design effects to generate variances [10]. For NHANES II and HHANES data, a single, average design effect was used to modify the subgroup variance calculated under the assumption of simple random sampling, because variance estimates calculated for subgroups by the Taylor series linearization method tended to be unstable for HHANES data [11]. The average design effects used for mean intakes of the various nutrients and food groups ranged from 1.3-2.8 for NHANES II and 1.0-1.96 for HHANES.

All statistics were calculated using SAS [12]. Means and prevalences were compared between population groups within an age/sex category with a t-test. Because multiple comparisons were made, the critical t value was calculated using the Bonferroni approach [13]. In order to limit the total number of comparisons, only differences between NHB and the other population groups were tested for statistical significance. Thus, differences between NHW and the Hispanic groups, or between Hispanic subgroups, were not tested statistically.

Table 2. Mean dietary nutrient intake* by race, sex, and age: NHANES II, 1976-80

Dietary variable, sex, and age	Non-Hispanic Black	Non-Hispanic White
MALES		
Energy (kcal/day)		
2-6 y	1542±27[a]	1673±15[a]
18-44 y	2530±113	2764±43
65-74 y	1615±62[a]	1853±21[a]
Dietary iron (mg/day)		
2-6 y	9.7±.23[a]	10.7±.12[a]
18-44 y	15.1±.70[a]	17.4±.30[a]
65-74 y	11.5±.40[a]	14.5±.22[a]
Dietary vitamin C (mg/day)		
2-6 y	101± 5	102±2
18-44 y	118±12	107±4
65-74 y	85± 7	102±2
FEMALES		
Energy (kcal/day)		
2-6 y	1581±33	1546±14
18-44 y	1563±61	1635±22
65-74 y	1192±37	1307±14
Dietary iron (mg/day)		
2-6 y	10.6±.37	9.5±.11
18-44 y	9.8±.43	10.9±.17
65-74 y	8.9±.30[a]	10.4±.14[a]
Dietary vitamin C (mg/day)		
2-6 y	97± 5	97±3
18-44 y	91±10	88±3
65-74 y	113± 8	104±2

*Means ± standard error of mean; means bearing common superscripts within an age group differ significantly, $p < 0.05$.

Results

A. Nutrient and food group intake

Mean nutrient intakes from food are shown by population group, sex and age in Table 2. Among males, NHB consumed less energy and iron than NHW. When expressed as iron density (iron per 1,000 kcal), iron intakes among males did not differ between races (data not shown). Vitamin C intake by males was similar in the two groups. Among females, there was a tendency for adult NHB to consume less iron than NHW females, although the difference was statistically significant only for the elderly females. Females of both races consumed similar amounts of energy and vitamin C. Differences in iron intake between NHB and NHW remained after stratifying by poverty status for both sexes (data not shown).

Mean frequency of food group consumption is shown by population group and age for males in Table 3 and for females in Table 4. Differences between NHB and the other population groups in meat consumption were not consistent across age groups. NHB tended to report more frequent intake of meat than PR in all age/sex categories. NHB children of both sexes tended to consume meat more frequently than children in the other population groups. Elderly NHB males and females consumed meat less frequently than NHW. NHB in all age and sex groups consumed poultry and organ meats more frequently than NHW. NHB also consumed organ meat more frequently than Cubans and PR, although the differences were statistically significant only among females. Fish consumption tended to be higher among NHB than MA or Cubans for elderly females and males of all ages.

NHB reported less frequent intake of mixed dishes than NHW (all age and sex groups) and MA and PR (children and young adults). Differences between NHB and the other population groups in cereal consumption were not consistent across age groups. NHB reported more frequent intake of cereal than MA among children and Cubans among

Table 3. Mean food group consumption* among males by population group and age: NHANES II, 1976-80 and HHANES 1982-84

Food group and age	Non-hispanic black	Non-hispanic white	Mexican American	Cuban	Puerto Rican
	Times per week consumed				
Meat					
2-6 y	7.9^{abc}	6.6^{a}	6.2^{b}	7.0^{+}	5.9^{c}
18-44 y	7.1	7.5	6.9	6.8	5.6
65-74 y	4.3^{a}	5.6^{a}	4.6	4.9^{+}	3.8^{+}
Poultry					
2-6 y	1.9^{a}	1.3^{a}	1.6	1.9^{+}	2.3
18-44 y	1.9^{a}	1.3^{a}	1.6	1.7	2.2
65-74 y	1.8^{a}	1.2^{a}	1.4	2.1^{+}	1.8^{+}
Organ meat					
2-6 y	0.4^{a}	0.2^{a}	0.4	0.2^{+}	0.3
18-44 y	0.5^{a}	0.3^{a}	0.5	0.3	0.3
65-74 y	0.5^{a}	0.3^{a}	0.4	0.2^{+}	0.2^{+}
Fish					
2-6 y	0.9^{a}	1.0	0.8	0.6^{a+}	1.0
18-44 y	1.2^{a}	1.0	0.8^{a}	1.0	0.9
65-74 y	1.0^{a}	0.9	0.6^{a}	0.7^{+}	0.6^{+}
Mixed dishes					
2-6 y	1.4^{abc}	1.8^{a}	2.8^{b}	2.2^{+}	3.0^{c}
18-44 y	1.2^{abc}	1.8^{a}	3.0^{b}	1.9	2.6^{c}
65-74 y	0.7^{ab}	0.9^{a}	1.1	1.5^{b+}	0.9^{+}
Legumes					
2-6 y	1.5^{ab}	1.4	3.9^{a}	1.6^{+}	3.0^{b}
18-44 y	1.5^{ab}	1.7	4.3^{a}	2.2	3.1^{b}
65-74 y	1.3^{a}	1.5	4.4^{a}	1.7^{+}	3.3^{+}
Cereal					
2-6 y	5.4^{a}	5.1	4.0^{a}	6.5^{+}	4.8
18-44 y	1.7^{a}	1.9	1.2	0.9^{a}	1.5
65-74 y	2.4^{ab}	3.5^{a}	2.0	1.8^{+}	1.2^{b+}
Grain					
2-6 y	13.4^{a}	13.0	17.7^{a}	11.4^{+}	11.5
18-44 y	12.3^{a}	12.5	17.3^{a}	11.0	10.7
65-74 y	12.0	13.6	15.2	12.2^{+}	9.6^{+}
Fruits and vegetables					
2-6 y	15.5^{a}	16.1	12.8^{a}	13.2^{+}	12.7
18-44 y	11.1	12.3	9.3	10.5	9.5
65-74 y	10.0^{a}	13.4^{a}	8.4	11.0^{+}	9.5^{+}

*Means bearing common superscripts within an age group differ significantly, $p < 0.05$.
+Statistic may be unreliable due to small sample size.

Table 4. Mean food group consumption* among females by population group and age: NHANES II, 1976-80 and HHANES 1982-84

Food group and age	Non-hispanic black	Non-hispanic white	Mexican American	Cuban	Puerto Rican
	Times per week consumed				
Meat					
2-6 y	7.3^{a}	6.8	6.4	6.8^{+}	5.1^{a}
18-44 y	6.4^{a}	6.1	5.7	5.5	4.6^{a}
65-74 y	4.0^{ab}	5.1^{a}	3.5	4.2	2.4^{b}
Poultry					
2-6 y	1.7^{ab}	1.2^{a}	1.7	2.3^{+}	2.4^{b}
18-44 y	1.8^{a}	1.3^{a}	1.6	2.0	2.2
65-74 y	2.2^{ab}	1.4^{a}	1.5^{b}	2.2	2.0
Organ meat					
2-6 y	0.1^{ab}	0.2^{a}	0.3	0.2^{b+}	0.3
18-44 y	0.5^{abc}	0.3^{a}	0.4	0.3^{b}	0.3^{c}
65-74 y	0.4^{abc}	0.3^{a}	0.5	0.2^{b}	0.2^{c}
Fish					
2-6 y	0.9	0.9	0.9	0.6^{+}	1.1
18-44 y	1.1	1.1	0.9	1.0	1.0
65-74 y	1.0^{ab}	0.9	0.6^{a}	0.6^{b}	0.8
Mixed dishes					
2-6 y	1.3^{abc}	1.7^{a}	2.9^{b}	1.9^{+}	2.7^{c}
18-44 y	1.1^{abcd}	1.6^{a}	2.5^{b}	1.9^{c}	2.5^{d}
65-74 y	0.6^{a}	0.9^{a}	1.0	1.0	1.1
Legumes					
2-6 y	1.7^{ab}	1.4	3.7^{a}	1.8^{+}	3.2^{b}
18-44 y	1.4^{ab}	1.2	3.7^{a}	1.9	2.7^{b}
65-74 y	1.1^{a}	1.1	3.5^{a}	0.8	1.8
Cereal					
2-6 y	5.1^{a}	5.2	3.9^{a}	3.6^{+}	4.7
18-44 y	1.9^{a}	1.9	1.7	1.1^{a}	1.8
65-74 y	2.7^{a}	3.4^{a}	2.4	2.0	2.9
Grain					
2-6 y	13.9^{ab}	13.1	17.9^{a}	13.9^{+}	11.8^{b}
18-44 y	11.0^{a}	10.3	16.8^{a}	11.9	9.7
65-74 y	11.8^{a}	12.2	14.7	10.3	8.7^{a}
Fruits and vegetables					
2-6 y	14.4^{a}	15.5	13.2	14.0^{+}	11.6^{a}
18-44 y	13.1^{a}	13.3	11.0	10.4	9.5^{a}
65-74 y	12.5^{a}	16.1^{a}	10.4	11.8	9.8

*Means bearing common superscripts within an age group differ significantly, $p < 0.05$.
+Statistic may be unreliable due to small sample size.

young adults but less frequent consumption than NHW in the elderly.

MA and PR ate legumes more frequently than NHB in all age and sex groups. MA also consumed grain products more frequently than NHB, whereas PR consumed these products less frequently. PR also consumed fruits and vegetables less frequently than NHB, although the difference reached statistical significance only among females.

B. VMS Use

Fewer NHB than NHW used VMS in all age and sex groups (Table 5). VMS use among Hispanics of both sexes tended to be intermediate between that reported by NHW and NHB. Use of vitamin-C containing supplements was more common than iron- containing products in all three population groups examined. Not surprisingly, differences between population groups in the use of iron or vitamin C products paralleled those seen for VMS of all types.

Median daily iron intakes from VMS among 18-44-year-old males and females who used an iron-containing product ranged from 18-27 mg in the 3 population groups, which corresponded to 100- 270% of the age and sex-specific Recommended Dietary Allowance (RDA) [14] for iron (data not shown). Median daily vitamin C intakes among 18-44 year-olds who used a product with this nutrient ranged from 90-180 mg; these values corresponded to 143—300% of the RDA for vitamin C. Because sample sizes were small, and because the analyses excluded persons whose median daily intake of vitamin C or iron was unknown, these data should be considered "provisional". It was not possible to examine the amount of iron and vitamin C consumed from VMS in the other age and sex groups due to inadequate sample sizes.

Differences in VMS use between population groups among 18-44- year-old males and females remained after stratifying by poverty status for all three types of VMS (Table 6); the differences were not statistically significant

among poor females, however. It was not possible to examine VMS use by poverty status in the other age categories due to inadequate sample sizes.

Discussion

Differences in all three aspects of dietary habits examined in this study were noted among the population groups. Many of the patterns observed in this study are consistent with patterns noted in other studies. For example, similar trends in iron intake from food between blacks and whites among adults were reported in the 1977-78 NFCS [15]. More recent data from the CSFII [16] suggest that these trends in iron intake by race have continued, at least among females.

The food frequency data provided insight about qualitative differences in dietary habits rather than quantitative differences, since the actual amount of food consumed was not determined. The most consistent differences in frequency of food group consumption between NHB and the other population groups occurred for poultry, organ meats, mixed dishes, legumes, and grains. Our finding of greater use of poultry and organ meats among NHB than NHW has been observed by others [17,18]. The greater frequency of consuming legumes and grain products by MA when compared to NHB likely reflects greater consumption of beans and tortillas in this group. While the frequency of legume consumption was greater among PR than NHB, the opposite was true for grain products, which may reflect substitution of starchy tubers like yams and tanier for grain products among PR.

The lower use of VMS by blacks and the lower use of iron- containing products compared to vitamin C-containing products in all three population groups is also consistent with other research [3,4]. The small sample size who reported using iron or vitamin C products among Hispanics prevented us from examining use of these specific VMS in the Hispanic subgroups. Moss et al [3] had previously reported that approximately 29% of adult PR used VMS of all types, compared to 25% of adult MA and 21% of adult Cubans.

Table 5. Vitamin-mineral supplement use by population group, sex, and age: NHIS, 1986

Age and type of supplement	Non-hispanic black	Non-hispanic white	Hispanic
	Percent*		
MALES			
2- 6 y			
All types**	30±4[a]	49±2[a]	35±4[a]
Iron product	8±2[a]	21±2[a]	16±3
Vitamin C product	27±4[a]	46±2[a]	33±4
18-44 y			
All types	19±3[a]	33±1[a]	27±4
Iron product	11±4	17±2	14±3
Vitamin C product	17±3[a]	30±1[a]	23±3
65+ y			
All types	10±4[ab]	31±2[a]	45±11[b†]
Iron product	3±2[a]	17±2[a]	13±10†
Vitamin C product	8±3[a]	28±2[a]	17±10†
FEMALES			
2-6 y			
All types	31±4[a]	46±2[a]	40±5
Iron product	17±4	18±1	19±5
Vitamin C product	29±4[a]	44±2[a]	39±5
18-44 y			
All types	27±2[a]	43±1[a]	28±3
Iron product	15±2[a]	26±1[a]	16±3
Vitamin C product	20±2[a]	35±1[a]	20±3
65+ y			
All types	17±3[a]	45±2[a]	36±8†
Iron product	11±3[a]	22±1[a]	12±7†
Vitamin C product	13±3[a]	35±2[a]	30±9†

* Percents ± standard error; percents bearing common superscripts within an age group differ significantly, p< 0.05.
** Any vitamin, mineral, or fluoride product.
† Statistic may be unreliable due to small sample size.

Table 6. Vitamin-mineral supplement use among 18-44-year-old persons by poverty status, population group, and sex: NHIS, 1986.

Sex and type of supplement	Poor NHB**	Poor NHW**	Poor Hispanic	Nonpoor NHB	Nonpoor NHW	Nonpoor Hispanic
	Percent					
MALE						
All types***	9[a]	27[a]	8†	22[a]	34[a]	34
Iron product	3[a]	16[a]	3†	14	17	19
Vitamin C product	8[a]	26[a]	7†	21[a]	31[a]	28
FEMALE						
All types	21	34	18	31[a]	44[a]	32
Iron product	11	18	12	19[a]	28[a]	17
Vitamin C product	14	27	12	26[a]	37[a]	22

* Percents bearing common superscripts within a poverty group and product type differ significantly, p< 0.05.
** NHB=non-Hispanic black; NHW=non-Hispanic white.
***Any vitamin, mineral, or fluoride product.
† Statistic may be unreliable due to small sample size.

Some apparently large differences in mean intakes or VMS use between population groups were not statistically significant in this study. Our ability to detect statistically significant differences between population groups was hindered by the relatively large standard errors associated with many of the point estimates for NHB and Hispanics. The large standard errors for these ethnic groups likely resulted from the relatively small sample sizes of these groups included in the three surveys.

Comparison of food group intake between NHB from NHANES II and MA, Cubans, and PR from HHANES could be confounded by differences in dietary assessment methods or secular trends in dietary intake between

the two surveys. For example, the food frequency was less detailed in NHANES II than in HHANES, so some of the food categories in HHANES had to be combined to form comparable categories. This might obscure differences within a food group. In addition, the two surveys were conducted at different times, so that secular trends in food group intake in the population or in population subgroups could have occurred.

Determining whether the iron intakes reported by NHW and NHB in this study are adequate is difficult due to the variability of iron requirements among individuals and to homeostatic mechanisms that regulate iron absorption based on body iron stores. Comparing mean intakes with the RDA can lead to erroneous estimates of the prevalence of inadequacy [19]. For example, the prevalence of iron deficiency among women of child-bearing age in the population was much lower than would be predicted from the prevalence of low iron intake in that group [16]. The differences in dietary iron intake between NHB and NHW are consistent with the slightly higher prevalence of impaired iron status observed among blacks in previous analyses of NHANES II data [15,20]. However, dietary iron intake was not associated with iron status in previous studies using NHANES I and NHANES II data except among the youngest children [16,21]. Demonstrating a correlation between dietary intake and biochemical indicators of iron status with the HANES data currently available is complicated by a number of measurement factors, including lack of information about the amount of iron contributed from VMS, lack of information about the bioavailability of the iron consumed, and the within-person variation in dietary intake data based on a single 24-hour recall. The occurrence of a relationship between intake and status among infants and young children in previous studies using HANES data has been attributed to the greater uniformity of their diet over time, so that the single 24 hour recall likely provides a reasonable estimate of usual intake [16].

A more in-depth assessment of the impact of these measurement issues on the ability to detect a relationship between intake and status will be possible with data from the third National Health and Nutrition Examination Survey (NHANES III), which began in 1988 and will be completed in 1994. The dietary component of NHANES III includes replicate 24-hour recalls from a subsample, which will allow us to assess the amount of within-person variance in the dietary data, and also allows the possibility of statistically adjusting the nutrient intake distribution to reduce the effect of within-person variance [19]. Quantitative nutrient information from VMS is also being collected, so better estimates of total iron intake will be available. The structure of the food composition database will permit use of models to estimate bioavailable iron [22,23]. Finally, NHANES III will collect data on dietary intake and biochemical indicators of iron status simultaneously from representative samples of NHW, NHB, and MA, so that differences in methodology or secular trends between surveys will not confound interpretation of differences between the population groups.

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IRON DEFICIENCY DIAGNOSIS

Francisco Chew
Institute of Nutrition of Central America and Panama,
Guatemala, C.A.

Magnitude of the Problem

Iron deficiency is the most common specific nutrient deficiency. The global prevalence of anemia in the world is close to 30%, so that according to information from 1980, 1.3 billion of persons are anemic. In developing countries, the prevalence is approximately 36% and in developed countries, it is close to 8% (1). The great majority of anemias are due to iron deficiency.

Iron deficiency is most prevalent in small children, adolescents, women of reproductive age and during pregnancy. The global prevalence for children is 43% and for pregnant women it is 51% (1).

The regions with higher prevalence of anemia are Africa and South Asia. In Latin America, almost 30% of adult males and 30% of pregnant women are anemic.

In Europe, prevalence for adult males is 10% and for pregnant women it is 14% (1). In the United States 25% of infants and 10% of pregnant women have some degree of anemia (2).

Biological importance

Iron is found in every cell of the body and plays an important role in many biochemical reactions. Its main function in the heme molecule is oxygen transport. It is also part of many enzymes in the mitochondrial electron transport system. Consequently, iron deficiency produces metabolic and enzymatic impairments. When such deficiency is severe, it causes anemia.

There are several causes of iron deficiency. Children and women are specially affected because of their relatively high iron requirements. The amounts needed to meet the requirements for fetal and childhood growth, for blood volume expansion during pregnancy and for replacement of menstrual blood losses, frequently exceed the amounts of iron in the diet. In adults with iron deficiency anemia, one should also suspect a pathologic blood loss, like in tumors or gastrointestinal ulcers, and iron malabsorption caused by enteropathies or post-gastrectomy.

In developing countries, iron bioavailability from the diet is usually low, mostly due to a low content of substances that enhance iron absorption. Frequent pregnancies and an increased loss of blood caused by intestinal parasites, mainly hookworms, contribute to the anemia.

Physiological distribution in the body

Iron-containing compounds in the body have been classified in two categories (3), namely those that have a metabolic or enzymatic function, and those that are associated with iron transport and storage. Compounds in the first category, also called functional or essential ferric compounds, are mostly heme and protein complexes. They include hemoglobin, myoglobin, cytochromes, and granulocyte myeloperoxidase. Enzymes with non-heme iron such as the metaloflavoproteins, also belong in this category. Other enzymes have iron as a cofactor, such as phosphoenolpyruvate carboxykinase and tryptophan pyrrolase (4).

The second category of iron compounds include ferritin and hemosiderin. They are found mainly in the liver, reticuloendothelial cells and bone marrow (5).

Iron deficiency stages

Iron deficiency anemia is the final result of a series of sequential changes that occur in iron depletion. There is a progression from an adequate iron state to iron deficiency ane-

mia, in three overlapping stages (3). The first stage consists of depletion of iron stores in liver, spleen and bone marrow. This stage is characterized by a decrease in the concentration of serum ferritin.

In the second stage there is a reduction in iron transport, characterized by an increase in the iron binding capacity of serum and a decrease in serum iron concentration. These changes result in a reduction of transferrin saturation, calculated as:

$$\frac{\text{Serum iron}}{\text{TIBC}} \times 100 = \%\ \text{transferrin saturation}$$

The third stage occurs when iron delivery decreases to such extent that it limits the production of hemoglobin. This stage is characterized by an increase in free erythrocyte protoporphyrins, anemia and microcytosis. This third stage can be defined as a decrease in hemoglobin concentration below 95% of the reference standards for a given age and gender group.

Causes of iron deficiency

A low hemoglobin concentration in iron deficiency is the result of an imbalance between iron absorption and body requirements. This can be due to one or more of the following:

- poor nutrient intake
- poor absorption
- poor utilization
- increase in demands
- increase in nutrient losses

Poor nutrient intake

In developed countries, a diet poor in iron is a very unusual cause of iron deficiency in adults (6). Men and non-menstruating women should not have much difficulty in obtaining the necessary iron from their usual diet. For example, in the United States, diets provide between 12 and 18 mg Fe/day.

Iron balance can be impaired when adolescents and menstruating women consume inadequate, restrictive, diets with poor iron contents, as low as 10 mg per day or less (6). In some social groups, diets consumed by women provide less iron than those consumed by men, probably because of lower energy intakes. Food taboos, specially during pregnancy and puerperium, contribute to a low iron intake.

In developing countries, economic factors limit the intake of meat and foods rich in ascorbic acid, which stimulates iron absorption.

Poor absorption

The poor absorption of iron from cereals, legumes, fruits and vegetables is well known. These are frequently the only sources of dietary iron in population groups of low socioeconomic level. The absorption of non-heme iron is highly variable and depends on the nature of other components of foods. In some countries it has been observed that iron bioavailability from the diet is low, due to an imbalance between the different dietary factors that promote or inhibit its absorption. Iron absorption can improve in a diet with low bioavailability when there is an increase in the intake of foods that promote iron absorption, such as foods rich in ascorbic acid.

Iron absorption usually is inadequate in chronic diarrhea and after partial or total gastrectomy.

Poor utilization

There are some conditions where iron availability for hemoglobin synthesis is decreased despite normal or high body iron stores. These include inflammatory processes that impede mobilization of iron from the storage cells of the reticuloendothelial system (7).

Increase in demand

Rapid growth rate and an increase in red cell mass are the most important factors for the occurence of iron deficiency in children between 6 months and 3 years of age. Premature infants and twins, are high risk groups to develop iron deficiency anemia, unless supplementary iron is given. This is because their iron stores at birth are smaller and their weight gain is proportionally greater than in single babies born at term.

When iron stores decrease to allow

hemoglobin synthesis, dietary factors gradually play an important role to avoid or produce deficiency. Between 3 years of age and adolescence, iron deficiency is less frequent because growth rate decreases and older children consume a more varied diet. Nevertheless, in developing countries, where hookworm infections are common, the prevalence and severity of iron deficiency are high (8). Adolescents are another high risk group, due to their rapid growth. Hemoglobin concentration increases between 0.5 and 1.0 g/dl/year (3). Menarche, which usually follows or accompanies this rapid growth, requires additional iron to balance menstrual iron loss. Furthermore, although dietary iron content per 1000 kcal is similar in men and women, energy intake is lower in women. This results in lower iron intake compared to men.

Pregnant teenagers, have an even greater risk of developing iron deficiency, because pregnancy usually begins with low or depleted iron stores due to the concurrent demands for rapid growth. Pregnant women have increased requirements of nutrients, including iron. This mineral is needed to expand the maternal red cell mass and to meet the requirements of the fetus (9). Lactating women have lower iron requirements than menstruating women due to post-partum amenorrhea.

Increase in iron losses

The two most common causes of iron deficiency in adults are an increase in menstrual bleeding and visible gastrointestinal bleeding (6). Bleeding from the nose, mouth, genitourinary tract and hemorrhoids, can also produce an important increase in iron losses.

Other important causes of chronic blood loss include the use of intrauterine devices (10), vascular anomalies and occult gastrointestinal bleeding due to a peptic ulcer, hiatus hernia, aspirin intake, tumors and intestinal parasites.

Long distance runners can also have blood losses due to mechanical trauma of the erythrocytes, with iron loss through urine (11).

Diagnosis of iron deficiency

Biochemical indicators: Some diseases and nutrient deficits are diagnosed and easily detected when fully developed, but during their initial stages or when the expression is mild, diagnosis becomes very difficult. Iron is not the exception (6).

It would be desirable to classify the different laboratory tests according to the iron deficiency stage, but in practice, it has been found that laboratory results are not consistent with the degree of iron deficiency, especially in those who have mild anemia (12). In contrast, severe iron deficiency anemia is characterized by hypochromia, microcytosis, high values of TIBC and free protoporphyrins in erythrocytes, and low levels of serum iron, transferrin saturation and plasma ferritin. Laboratory tests used to diagnose iron deficiency can be classified as screening tests and confirmatory tests. Screening tests are commonly used for initial evaluation of populations or groups that have a high probability of iron deficiency.

Screening tests

Blood hemoglobin concentration: This is the most widely used test for screening of anemia and iron deficiency. The blood extraction technique and the exact dilution of the sample with anticoagulant are extremely important. There is evidence that hemoglobin concentrations differ between venous and capillary blood samples (13).

For appropriate interpretation of hemoglobin concentrations, reference standards must be used that account for sex, age and altitude above sea level (14). It is recommended that anemia should be considered to exist in those whose hemoglobin levels are lower than the figures given below. It is necessary to remember to make corrections of hemoglobin concentration for altitude.

HEMOGLOBIN LEVELS INDICATIVE OF ANEMIA IN POPULATIONS LIVING AT SEA LEVEL

AGE/SEX GROUP	HEMOGLOBIN LEVEL (g/dL)
Children 6 months - 5 years	< 11
Children 6 - 14 years	< 12
Adult males	< 13
Adult females (non-pregnant)	< 12
Adult females (pregnant)	< 11

Packed red blood-cell volume or hematocrit: This is determined by centrifuging a blood sample collected in a heparinized tube. There are convenient micromethods for use with a finger prick. When comparing mean values of large populations, hematocrit can be considered equivalent to hemoglobin concentration multiplied by 2.9 (8). Hematocrit is not as sensitive as hemoglobin in the diagnosis of anemia, especially in children under 2 years of age, where the percentage of false negatives is 62% compared with 43% with hemoglobin (15). This is due to the fact that hypochromia, which is measured by hemoglobin concentration, precedes the reduction in cell size.

Mean corpuscular volume and other hematologic indices

This hematologic index is derived from the relation of hematocrit and the red cell count. At present, with the presence of electronic counters, MCV determination is a practical examination. Microcytosis, characteristic of iron deficiency, is evidenced by a low MCV. Many other anemias are characterized by a normal or increased value of MCV. A low hemoglobin with a low MCV is almost diagnostic of iron deficiency anemia, but differential diagnosis from Thalassemia Minor and the anemia found in infections and chronic diseases must be considered. As is the case for hemoglobin and hematocrit, MCV normally changes during development, and comparison with age-specific reference standards is necessary.

Other hematologic indices are Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). Combined with MCV, they allow differentiation of anemias as micro-, normo- and macrocytic, and as hypo- or normochromic.

A peripheral blood smear also allows distinguishing the microcytosis and hypochromy of iron deficiency.

Confirmatory tests

Plasma or Serum Ferritin Concentrations:

Determination of plasma or serum ferritin concentrations can be done by radioimmunoassay or ELISA. It permits an estimate of body iron stores. Ferritin concentration allows the evaluation of iron nutritional status, both within the normal range or in conditions of iron deficiency or excess. Nevertheless, since it is an acute phase protein, iron-deficient persons can have false normal values of ferritin if they have an infection, inflammatory process or liver disease (16-17).

Figure 1 shows the variations in ferritin concentration related to age (3). High values are found in the newborn, but they decrease rapidly during infancy and remain low. In males, there is an increase after adolescence but in females the increase occurs after menopause. At any given age, a ferritin value below 10 µg/liter indicates depletion of iron stores.

Serum Iron Concentration:

This is a spectrophotometric technique that measures free serum iron after being colored. When manually determined, erroneous results produced by environmental contamination may occur. Other limitation is the circadian variation of the serum iron values after the age of 3 years (18). Its concentration decreases in iron deficiency. Infections also produce a sharp decrease.

Serum Total Iron-Binding Capacity (TIBC)

This is a measure of the capacity of circulating transferrin to bind and transport iron. The normal range is between 250 and 400 µg/dl (45-72 µmoles/liter). TIBC is increased

in iron deficiency.

Transferrin Saturation:

This is an estimate of the relative amount of transferrin that is available to transport iron. In iron deficiency, most of the transferrin is not bound to the mineral. Thus, transferrin saturation is low. It is calculated from the relation of serum iron and TIBC. In adults, a transferrin saturation below 16% is considered as iron deficiency and in children the value is below 10% (19). Transferrin saturation decreases in infections, mainly due to an artificial reduction in the circulating levels of iron.

Free Erythrocyte Protoporphyrin Concentrations:

When there is not much iron available for combining with protoporphyrin IX to form heme, free protoporphyrin accumulates in the erythrocyte. This measurement has the advantage of not being affected by recent iron medication, as is the case for serum iron and ferritin after the intake of high doses of iron (20). The normal upper limit of protoporphyrin is 3 µg/g Hb (30 µg/dl of total blood or 100 µg/dl of erythrocytes). In mild iron deficiency it seldom increases to twice the upper limit. The free erythrocyte protoporphyrin concentration distinguishes iron deficiency anemia from Thalassemia Minor, as it is elevated only in the former.

Infection and lead poisoning also increase free erythrocyte protoporphyrins. When we find a hemoglobin concentration or hematocrit below the normal level, the diagnosis of iron deficiency anemia should be considered, but the chances increase if we also find a low or a lower normal value of MCV. If the anemia is mild and iron deficiency is suspected but not confirmed, there are two alternatives for diagnosis. We either carry out additional laboratory tests (ferritin, transferrin saturation, free erythrocyte protoporphyrin) or a therapeutic trial with iron.

Therapeutic Diagnostic Trial:

A short-term therapeutic trial with iron for a month can be justified when anemia is diagnosed and no other laboratory tests are available. An adequate hemoglobin response to treatment with iron, arbitrarily fixed as an increase of 1 g/dl or more after a period of 30-45 days, permits the recognition of individuals whose initial hemoglobin values, despite being in the normal range, are low for them. If there is an increase in hemoglobin concentration, medication should be continued for 3 to 5 months to replenish iron stores. The dose usually used for the trial is 60 mg iron/day in adults and 3 mg/day in children.

Other causes should be explored if there is no response after one month treatment. The decision on how to proceed will depend on the post-treatment hemoglobin concentration compared to the reference standards. If anemia persists, a more extensive diagnostic search should be carried out.

Differential diagnosis:

Two sets of conditions require clarification through differential diagnostic procedures. One is to differentiate between the anemia and the microcytosis that characterizes iron deficiency, Thalassemia Minor and other hemoglobinopathies. The other is to confirm iron deficiency under conditions that might confuse the interpretation of laboratory results.

Thalassemia Minor: After iron deficiency, Thalassemia Minor is the most common cause of mild anemia and microcytosis. Alpha thalassemias are not frequently diagnosed. Approximately 28% of North American blacks do not have one of the four alpha globin loci and almost 3% lack two of these genes (21,22). More than 25% Indochinese who live in North America also have some type of alpha thalassemia (23).

In Thalassemia Minor, MCV is below normal, while hemoglobin concentration is within the lower normal limit or slightly lower (24). An abnormally low MCV with normal hemoglobin suggests Thalassemia Minor, although it should be kept in mind that an individual with Thalassemia can be anemic (i.e., with subnormal hemoglobin concentration). Alpha Thalassemia Minor should be suspected if one of the patient's parents also presents microcytosis.

The index of Mentzer (25) is useful to dif-

ferentiate iron deficiency from Thalassemia Minor. It is calculated from the relationship of MCV to erythrocyte count, in millions. In Thalassemia Minor, the erythrocyte count tends to be higher for the degree of anemia present. An MCV/RBC of 13 or less is found in 85% of individuals with Thalassemia Minor, while a similar percentage of patients with iron deficiency anemia have higher values.

In beta Thalassemia Minor, hemoglobin electrophoresis reveals an elevation in hemoglobin A2. When iron deficiency anemia coexists with beta Thalassemia Minor, hemoglobin A2 is frequently normal.

Chronic inflammatory diseases:

Chronic diseases are commonly associated with mild anemias, either due to iron deficiency or other causes (26). The factors that predispose to anemia include a decrease in erythrocyte lifespan and a weak erythropoietic response to anemia. There can be changes in iron metabolism that include a lower reutilization of lysed red cells and lower intestinal absorption of iron. Both changes lead to a redistribution of hemoglobin iron towards body stores and contribute to a decrease in serum iron concentration.

Some medications used in chronic inflammatory diseases can worsen the anemia. For example, aspirin can produce occult gastrointestinal bleeding (8). Chronic inflammation makes the diagnosis of iron deficiency difficult. Both conditions are characterized by mild anemia, elevated free erythrocyte protoporphyrins and low serum iron. MCV is occasionally diminished in inflammatory conditions, while TIBC may be diminished and serum ferritin may be normal or high in chronic diseases (6). A special problem is the interpretation of serum ferritin concentration in the diagnosis of iron deficiency in patients who suffer rheumatoid arthritis, chronic renal disease, lymphomas, leukemias and other cancers. Ferritin concentration is usually increased in these conditions. A therapeutic trial with iron for a month could be more useful than other laboratory tests to identify the cause of mild anemia in chronic diseases. The therapeutic trial may diminish the anemia (8), since some patients respond to iron therapy, despite having serum ferritin concentrations as high as 50 µg/liter.

Acute Infections:

Acute infections produce tissue sequestration of some nutrients, among them, iron. Laboratory results in acute infection, are similar to those in chronic inflammatory disease. There is an abrupt decrease in serum iron and transferrin saturation, and increases in erythrocyte protoporphyrin and serum ferritin (27).

Combined nutritional deficiencies

In developing countries, iron deficiency often occurs with other nutritional problems, such as protein-energy malnutrition, folate and vitamin A deficiencies. The diagnosis of iron deficiency in some of these multiple deficiency states may not be simple.

Protein-Energy Malnutrition:

Anemia, defined as a low blood hemoglobin concentration, is a common finding in patients with severe protein-energy malnutrition. These patients usually have hemoglobin values below 10 g/dl. However, this hemoglobin concentration is usually sufficient to allow adequate tissue oxygenation, due to the decreased body mass and low physical activity that are part of this syndrome. When these patients start increasing body mass and become more active as a result of nutritional rehabilitation, iron deficiency could lead to anemia with clinical or metabolic signs of hypoxia, due to insufficient iron stores for hemoglobin synthesis.

Folate Deficiency:

Combined iron and folate deficiencies are common, particularly in women during the second half of pregnancy. Children with diarrhea or malnutrition are also at risk of having folate deficiency (28).

In severe iron deficiency, folate deficiency may be hidden. In these cases, treatment with iron will stimulate erythropoiesis to an extent

where the body pool of folates becomes insufficient and folate deficiency anemia with macrocytosis may appear.

Vitamin A:

Studies in humans and laboratory animals have shown an interaction between the nutritional status of vitamin A and iron (29-32). These studies indicate that vitamin A deficiency may lead to mild anemia characterized by low serum iron and elevated levels of stored iron. Epidemiologic studies in children and adult support this concept (33). Nutritional interventions with vitamin A in humans show a positive effect on iron nutriture (34-36).

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DIETARY FACTORS INFLUENCING BIOAVAILABILITY OF DIETARY IRON

Eugene R. Morris

United States Department of Agriculture
Agricultural Research Service
Beltsville Human Nutrition Research Center
Beltsville, Maryland, 20705

The percentage absorption of ingested dietary iron is influenced by (a) the level of body iron stores, (b) the amount of iron presented to the mucosa and (c) bioavailability factors. The principle factor in body iron homeostasis is considered to be control of absorption of ingested dietary iron from the intestinal lumen. In the normal person the level of body iron stores and/or the rate of hematopoiesis through an as yet unidentified mechanism regulates what we may term the basal level of iron absorption for each individual. Magnusson et al. (1) showed that absorption of iron from a standardized dose of ferrous ascorbate is inversely related to iron stores as measured by either serum ferritin concentration or stainable bone marrow hemosiderin. Furthermore, absorption of iron from this reference dose was 40% in borderline-iron deficient individuals (serum ferritin of 30 ng/ml). Within certain bounds determined by the basal level of iron absorption, percentage absorption will vary inversely with the amount of iron presented to the intestinal mucosa which regulates the amount of iron absorbed (2). This factor is more critical when dealing with therapeutic levels of iron supplements, however, and likely has little influence on the usual amounts of iron consumed in individual meals. Absorption of food iron ingested in individual meals can be influenced by bioavailability factors contained in the foods ingested at the same time. Layrisse et al. (3), for example, showed that iron from animal food sources was absorbed to a greater extent than was the iron of plant derived foods. This discussion is concerned with these bioavailability factors and will stress human studies. A large body of literature on animal and in vitro studies is available for interested parties.

The development of sensitive counting techniques has enabled use of radioiron labeled foods in iron absorption tests (3) in which absorption is calculated on the basis of the quantity of radiolabeled iron incorporated into circulating hemoglobin. Such techniques provided greater sensitivity and more precise measurement than is possible with traditional balance techniques. The early studies were conducted with single foods intrinsically labeled by biological incorporation of the radioiron label. About 2 decades ago the miscible nonheme iron pool concept was developed and validated, making possible measurement of iron absorption from complete meals by the extrinsic label method (4-7), even a whole day's meals (8), with correction for nonmiscible iron content such as insoluble contamination iron (9). The pool concept was extended to heme iron as well (10). Using one iron isotope, 55Fe or 59Fe, in a labeled heme compound and the other as the extrinsic tag for nonheme iron, absorption of both the heme and nonheme iron components of the same meal can be determined.

Heme Iron

Food iron may be classified as heme or nonheme for discussion of bioavailability. Heme iron compounds include myoglobin and hemoglobin in animal tissues and the many iron-porphyrin enzymes found in living cells. Some uncertainty remains at present for the exact heme iron content of animal tissues, but heme iron is estimated to be no more than 10% of the usual daily dietary iron

intake, even for omnivorous diets (11). Experimental evidence indicates that heme iron is absorbed from the intestinal lumen as the intact iron-porphyrin complex and the iron is then released in the mucosal cell (12). Factors identified as influencing heme iron bioavailability are the amount of heme iron consumed, the presence of meat (13) and possibly soy-protein in the meal (14). Heme iron from animal tissues is considered to be highly bioavailable and with the above exceptions, absorption is not affected by bioavailability factors.

Nonheme Iron

The major fraction of dietary iron is nonheme and absorption is, however, influenced by bioavailability factors. Examples of nonheme iron forms are; ferritin (the iron storage protein in animal tissues), phytoferritin (found in plant tissues), phosvitin (the iron binding phosphoprotein of egg yolk), and inorganic iron fortification compounds. Bioavailability factors influencing the utilization of nonheme iron may be broadly classified as enhancers and inhibitors. Bioavailability of fortification compounds may be further influenced by their chemical form.

Enhancers of Nonheme Iron Absorption

The most potent enhancing factor is ascorbic acid. Cook and Monsen (15) titrated the effect of graded levels of ascorbic acid on absorption of 4 mg (0.08 mmol) of iron from a semisynthetic test meal. Iron absorption was increased 4-fold by inclusion of 100 mg (0.57 mmol) of ascorbic acid. Similar degrees of enhancement of iron absorption have been attained by proportional amounts of ascorbic acid and nonheme iron with maize meals containing ferrous sulfate (16) and fortified rice meals (17). Hallberg et al. (18) observed that the effect of ascorbic acid was greatest when given in meals of relatively low iron bioavailability. Martinez-Torres et al. (19) observed only a slight enhancing effect of ascorbic acid added to veal liver. Sayers et al. (20) noted low iron absorption from meals that contained cooked products in which the ascorbic acid had been destroyed. Likewise, iron absorption from meals held for a prolonged time on a warming pad decreased as the ascorbic acid content decreased (21).

Although ascorbic acid as either the crystalline compound or orange juice was thought to be equivalent (18,22), Ballot et al. (23) found that other organic acids in citrus juices may also promote iron absorption. These low molecular weight organic acids are a second group of enhancers. A water solution of ascorbic and citric acid produced greater increases in iron absorption than did a solution of ascorbic acid alone. Using a rice meal, Gillooly et al. (24) found that the addition of malic, tartaric or citric acids (low molecular weight organic acids found in vegetables) stimulated iron absorption. Addition of succinic acid improved utilization of iron from ferrous sulfate tablets in a Swedish study (25). Citrate is thought to be an important component of calcium-fortified orange juice and its effect on iron absorption (26). Derman et al. (27) studied iron absorption from maize and sorghum beers produced by native South African methods and concluded that both the alcohol and lactic acid content acted to promote iron absorption. Addition of absolute ethanol to test meals increased iron absorption by both control subjects and alcoholics in a study by Chapman et al. (28). On the other hand, iron absorption of ferrous sulfate added to red wine was very poor unless a large portion of the polyphenols (tannins) were removed prior to adding the iron salt (29).

The third enhancing factor is the "meat factor" related to the presence of meat, fish and poultry (MFP) in a meal. When beef, lamb, pork, fish or chicken was substituted for ovalbumin in a semisynthetic meal, iron absorption increased 2- to 4-fold compared to that of the basal meal (30). Conversely, if the beef in a standard meal was replaced on the same protein basis by ovalbumin, iron absorption decreased significantly. Reduction was also observed if the beef in the standard meal was replaced by milk, cheese or whole egg, indicating the meat factor effect does not reside in all animal proteins. Fish is low in iron content compared to the other animal

tissues that express the "meat factor" effect, but inclusion of fish in a meal of vegetables improves the absorption of the nonheme vegetable iron (31). Several additional examples of the "meat factor" effect will be cited in the following discussion of iron absorption inhibitors. The mechanism whereby the "meat factor" effect is exerted is uncertain. The amino acid cysteine will mimic the "meat factor" effect in certain conditions (32), but other amino acids may also play a role (33). The iron of isolated ferritin is of low bioavailability, but is improved significantly when ferritin is given with meat or liver (34). Bjorn-Rasmussen et al. (10) suggested that digestion products of meat, amino acids and peptides, are responsible for maintaining the heme moiety soluble and readily absorbable. Incorporation of bovine serum albumin into a maize meal increased iron absorption by only 10%, whereas the addition of beef resulted in a 3-fold increase (35). Thus, Hurrell et al. (35) suggest that the "meat factor" effect resides in specific animal tissues and is not a common property of animal proteins per se.

Inhibitors of Nonheme Iron Absorption

The list of inhibitors includes (a) metal binding ligands added during food processing, (b) phosphoproteins and phosphate, (c) tannins (polyphenols) and phytate, (d) soy protein and (e) calcium.

Synthetic chelators are often added during food processing to provide protection against oxidative deterioration of the food product. Ethylenediaminetetraacetate (EDTA) is such a chelator and at a molar ratio to iron of 2:1 will decrease iron absorption by as much as 50% (36). However, pre-formed sodium ferric EDTA, [NaFe(III)EDTA] is a more bioavailable form of iron (37) which has been studied as an iron fortificant and could serve as an efficacious iron enrichment compound for dietaries that include no additional EDTA or other exogenous chelators (38).

Phosvitin, a phosphoprotein, is the iron-binding protein of egg yolk. Chodos et al. (39) observed very low absorption of radiolabeled iron from eggs and concluded that they would be an inadequate iron source in the face of increased physiological need. In studies validating the extrinsic tag-miscible nonheme iron pool concept, Bjorn-Rasmussen et al. (5,6) also observed markedly lower absorption of radioiron from eggs compared to wheat. When the two foods, labeled by two different isotopes of iron, were consumed together, absorption of the two isotopes was equal or greater than egg alone, but less than the wheat alone. Callender et al. (40) also observed low bioavailability of egg iron, but demonstrated that orange juice improved iron utilization appreciably. Rat studies in our laboratory showed that egg yolk iron had a relative biological value (RBV) of 20 to 30 compared with the response to the reference ferrous sulfate as 100 (41). Ascorbic acid at a molar ratio to egg yolk iron of 20:1 in our rat model increased the RBV to 100.

Another phosphoprotein, milk casein, reduced iron absorption by almost 50% when substituted for egg albumin in a liquid-formula meal (42). In the same study, milk whey protein also depressed iron absorption. Whey protein is lower than casein in phosphorus content, but not as low as egg albumin. Free phosphate salts have been shown to decrease iron absorption (43).

One example of the effect of tannins (polyphenols) on iron absorption is the depression of iron absorption from red wine (29). Rao et al. (44) noted very low iron absorption, 0.8-0.9%, from meals based on millet or sorghum which contain tannins compared to 4-5% absorption from rice based meals. Gillooly et al. (24) found an inverse relationship between iron absorption and polyphenol content of vegetables such as spinach, lentils, beetroot greens and aubergine. The same investigators found that iron absorption was almost doubled when pearled sorghum (pearling removes the outer layers of the grain) was substituted for whole grain sorghum in meals (45). The pearling process reduced the tannin content by 96%. Tea contains tannins, and tea consumed with meals will significantly decrease iron absorption (46-48). Coffee also contains some polyphenols and when consumed with a meal will also decrease iron absorption, but

to a lesser degree than tea (48,49).

Mineral balance studies conducted by the British nutritionists McCance and Widdowson in the early 1940s implicated phytate in brown or whole meal bread as a naturally occurring compound which inhibits iron absorption (50). Iron balances of human subjects were always more positive when they consumed white bread compared to whole meal bread. When phytate was added to white bread, iron balance was decreased. The anion phytate, inositol hexaphosphate, occurs in all seeds. In human diets, the cereal grains and legumes are the major sources of phytate. In wheat, phytate is concentrated in the bran fraction. Bjorn-Rasmussen (51) found iron absorption decreased in a linear manner as increasing amounts of wheat bran were incorporated into white bread. Reinhold and coworkers (52) suggested that the bran fiber of wheat is an important component causing decreased iron balance.

By feeding whole bran one cannot delineate between the action of fiber and phytate on iron absorption. In 1976 we identified monoferric phytate in aqueous salt extracts of wheat bran and obtained physicochemical evidence that the major fraction of iron in wheat bran is monoferric phytate (53,54). For rats, monoferric phytate is a readily bioavailable iron source, even in the presence of an excess of phytate. (55). Insoluble iron phytates, di- or tetra-ferric phytate or a calcium-iron-phytate complex, however, are poorly utilized by rats (55,56). Studies with dogs and humans showed that the iron of monoferric phytate equilibrated with the miscible nonheme iron pool of meals (57,58). Dephytinized wheat bran in muffins depressed iron absorption in humans almost to the same extent as whole bran muffins in an extrinsic tag test meal study (58). In a balance study, iron balance was more positive when whole bran muffins were consumed than when dephytinized bran muffins were eaten (59). The dephytinized bran in both of these studies contained the same amount of total phosphorus as the whole bran, but the phytate phosphorus was present as phosphate ion. We recognized the fact that the phosphate ion may have affected the degree of iron absorption or balance. Phytate intakes considerably greater than the intake by most vegetarians did not alter iron balance in a second balance study in our laboratory (60). We seemed unable to confirm directly that phytate is highly detrimental to iron nutriture. Several studies do, however, continue the early implication that phytate will hinder iron utilization. The effect of pearling of the sorghum cited above (45) may be phytate related because not only tannin, but phytate content as well was lower in the pearled grain. Recently, Hallberg et al. confirmed the inhibiting effect of whole wheat bran on iron absorption (61) and demonstrated that small additions of phytate as sodium phytate to white flour rolls depressed iron absorption (62). Addition of 10 mg of phytate phosphorus (0.054 mmol of phytate) to rolls containing 4.1 mg (0.072 mmol of iron) reduced iron absorption to about 40% of the level in the absence of added phytate. Greater amounts of added phytate reduced iron absorption still further, but the rate of decrease was less. It is possible that an insoluble iron phytate is forming when sodium phytate is added in a dough, in which case the iron would be relatively unavailable on the basis of our rat study. They suggest that the comparison meal or diet should be phytate free since even the very low amounts of sodium phytate had a depressing effect on iron absorption. The meals and diets we used in the dephytinized bran studies were not phytate free, but we cannot rule out the possibility that a fiber component interacted with the iron in our studies. The Swedish investigators (63) have shown that hydrolysis products of phytate having four or fewer phosphates per molecule do not inhibit iron absorption in their test model and the pentaphosphate is less inhibitory than is the intact phytate (63).

The "meat factor" effect of several purified animal proteins was discussed above. Cook et al. (64) investigated the substitution of soy protein for egg albumin in the semisynthetic test meal and found that soy flour, textured soy flour and soy isolate all had an inhibitory effect on nonheme iron absorption. Early

studies with labeled single foods showed that the iron of soybean was absorbed to a greater degree than from wheat and several other plant foods (3). Inclusion of a soy product in a hamburger meal at a ratio of 2:1, meat to unhydrated textured soy flour, reduced the percentage of iron absorption by 50%. Hallberg and Rossander (65) also observed reduced percentage iron absorption when soy products replaced beef in a hamburger meal. Morck et al. undertook a study of ways to overcome the inhibitory effect of soy protein (66). Compared to the uncooked material, baking of a soy protein or whole soybeans about doubled iron absorption. The addition of beef to an all-soy meal also improved iron absorption. The greatest stimulation of iron absorption was attained by adding ascorbic acid. Derman et al. found a 2- to 3-fold increase in iron absorption from a liquid soy-formula by adding ascorbic acid at ratio to iron of 20:1 (67). Just as different preparatory methods may alter the effect of soy protein on iron absorption, the products themselves may have inherent differences that will alter their effect on iron absorption. We were unable to detect a detrimental effect of soy-extended ground beef on level of iron stores in a 6-month human study and iron absorption from test meals was greater with one soy-extended beef patty meal than from the ground beef control meal (68,69). Picciano et al. (70) reported the RBV of a soy beverage was 66%, soy flour 81% and soy concentrate 92% as efficient as ferrous sulfate for hemoglobin regeneration in a rat study. Soy products can be prepared with different phytate contents (71), but removal of phytate from soy flour did not alter the inhibiting effect of soy in Hallberg and Rossander's study (65). Beard et al. compared soybeans with low phytate and moderate phytate content and found no difference in iron absorption (72).

Other legumes have been found to be poor sources of dietary iron for humans. Mean percentage absorption of iron from black beans, lentils, mung beans and split peas made into soups ranged from 0.8 to 1.9% (73). Lupine was found to contain low amounts of polyphenols and no detectable phytate, but iron bioavailability was less than from soybean (74).

Tannins and phytate are components associated with the morphological parts of seeds making up a large part of the dietary fiber. As discussed earlier, differentiating between the action of fiber components and phytate or tannin is difficult when whole foods are tested. Tests of isolated or purified pectin or cellulose in meals, and apple pectin, guar gum, or gum tragacanth consumed with ferrous sulfate showed no or only slight reduction in iron absorption (45,75). However, hemicellulose and lignin did produce significant reduction in absorption from ferrous sulfate (45). Cook et al. (75) compared iron absorption from a meal high in food fiber, chiefly from vegetables, and equal in iron, macronutrients, mineral and ascorbic acid content to a low fiber meal and found that iron absorption was about 50% of that attained from the low fiber meal (75). Kelsay et al. (76) found no difference in iron balance of men consuming diets high in fiber from fruits and vegetables or low fiber diets in which fruit juices were provided, however, the low fiber diet was supplemented with an iron salt to provide similar intakes.

A semisynthetic meal of the same mineral and proximate composition as a standard meal of natural foods was used by Monsen and Cook (77) to investigate the role of inorganic salts in iron absorption. If the calcium or phosphate salt was added alone, iron absorption was equivalent to when no inorganic salts were added, but if added together, the percentage iron absorption was significantly reduced (78). Whittaker and Cook (79) tested iron absorption when calcium carbonate and ferrous sulfate were consumed with a hamburger meal at molar ratios calcium/iron of about 10 and 47. Iron absorption was reduced by about 25% and 47%, respectively, of the value obtained in the absence of added calcium. When administered in water, the added calcium had a negligible effect on iron absorption. The two studies suggest an interaction of calcium with the phosphorus of the meal to reduce iron absorption. Adult men

consumed menus of natural foods that supplied 18.5 mg (0.33 mmol) of iron and 1800 mg (2.7 mmol) of phytate daily, primarily from 32 g of wheat bran in muffins, in another balance study in our laboratory (80). When daily calcium intake was 570 mg (14 mmol) iron balance was 0.6 mg, but balance was -0.8 mg when calcium intake was 1570 mg (39 mmol). The added calcium for the high calcium intake was provided by calcium carbonate baked into the muffins. There was a concomitant increase in fecal excretion of calcium, iron and phytate with the high calcium intake and a complex of the three ions could very well have formed. Based on results of our rat experiment (56), we would not expect iron of such mixed complexes to be bioavailable to humans. The influence of calcium on iron absorption is of special concern because of the current popularity of calcium supplementation for prevention of osteoporosis. To this latter end, calcium fortified orange juice was introduced into groceries nationally. Calcium is apparently complexed with citrate, and possibly other organic acids in the juice, and does not interfere with iron absorption (26,81), at least in rats.

Another possible interaction is the effect of inorganic zinc on absorption of inorganic iron. The converse, effect of iron on zinc, has been demonstrated in man (82). Crofton et al. (83) observed no difference in the plasma tolerance curve of iron when 842 micromol (almost 5 times the RDA) was taken orally, alone or with 344 micromol of zinc (molar ratio zinc/iron = 0.4). The coadministration of zinc with iron at ratios zinc/iron of 1 reduced the plasma appearance of iron and at ratio of 4, the appearance curve was markedly reduced (83). The plasma tolerance test is considered an index of absorption, but the doses used in this study were greater than the usual dietary intakes and in the form of soluble inorganic salts. Whether high intakes of zinc from foods would inhibit dietary iron absorption is not known.

Thomson et al. (84) demonstrated that manganese and iron intestinal transport systems are apparently interrelated. Using perfused rat intestinal loops, high managanese concentration in the perfusate impeded iron transport from the intestinal lumen. Wheat bran contains high manganese concentration relative to the amounts in most foods. This may, therefore, be a factor in the inhibitory action of wheat bran on nonheme iron absorption.

Fortification Iron Absorption

The bioavailability factors discussed above influence the absorption of fortification iron. For example, absorption of iron added to Baladi bread, a traditional Egyptian flat bread baked from high extraction flour, as ferrous sulfate was 16% of the absorption from a traditional European white bread (85). The Baladi is higher in fiber and phytate content. However, the chemical form of the fortification iron also affects bioavailability. Using the extrinsic tag method, Cook et al. (86) found that absorption of iron as ferrous sulfate or hydrogen reduced iron baked into rolls was about equal, but as sodium iron pyrophosphate or ferric orthophosphate, absorption was only 0.1 or 0.33 as efficient, respectively, as from the ferrous sulfate. Rao et al. (87) also observed very low bioavailability of iron from sodium iron pyrophosphate from typical Indian meals. Uncertainty about the validity of the extrinsic tag as a measure of absorption from reduced iron prompted a cooperative study in which the radiolabel was incorporated into the product during the manufacturing process, making the label intrinsic in nature (88). Compared to ferrous sulfate in an extrinsic tagged farina meal, the RBV to humans was 0.25 for ferric phosphate and 0.75 for electrolytic reduced iron.

The need for chemical and oxidative stability in the fortified food and concomitant nutritional efficacy stimulated search for suitable iron compounds . Carbonyl iron possesses suitable stability properties and, being finely divided, was thought to be bioavailable. Studies with rats indicated that the RBV of carbonyl iron was comparable to ferrous sulfate (89), but absorption studies with humans using intrinsically tagged product showed the iron to be only 5-20% as

bioavailable as the native wheat iron (90). Hallberg et al. (91) report studies of a new complex ferric orthophosphate having higher absorbability than iron phosphates previously used and with suitable chemical properties for stability in flour. Absorption of iron from sodium iron EDTA (37,38) was discussed in a previous section. Sugar (92) and curry powder (93) have been suggested as possible vehicles for an iron fortification compound. Significant improvement in iron status of a target population was observed when the fortified curry powder was used under field conditions (94). Ferrous fumarate and ferrous succinate have been studied as fortification compounds for infant cereals and the iron was found to be absorbed better than from ferrous saccharate by adults (95). Infants absorbed nutritionally significant amounts of iron from a ferrous fumarate-fortified infant cereal (96).

Summary

In 1978 Monsen et al. (97) published a scheme for estimating absorbable nonheme iron in a meal, based on the amount of the enhancing factors, ascorbic acid and meat, fish or poultry in the meal. Different levels of absorption were suggested from each class of meal, high, medium or low bioavailability, for different levels of iron stores. In addition, heme iron absorption was factored in to permit estimation of total absorbable iron. This scheme was incorporated into the Recommended Dietary Allowances published in 1980 by the National Academy of Sciences (98). It has been computerized (99) and its principles applied in calculating absorbable iron from the USDA food consumption data for the United States (100). This model provides aid to dietitians in designing menus that use bioavailability influences to help provide a maximum amount of absorbable iron.

Examples of how bioavailability factors can be manipulated to provide maximum absorbable iron are found in the studies of European and North American omnivorous and vegetarian meals and Latin American-type meals by Hallberg and Rossander (101,102). Absorption of nonheme iron from the European- and North American-type meals varied directly with the presence and amounts of enhancing factors. Absorption from the Latin American meal of maize, rice and black beans was significantly improved by the inclusion of ascorbic acid and/or beef. They also demonstrated that the efficacy of a meal containing iron fortified foods could be improved by including foods that have an enhancing effect on iron absorption.

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MATERNAL IRON DEFICIENCY AND PREGNANCY OUTCOME

Bonnie Worthington-Roberts

Department of Nutritional Sciences
and Child Development Center
University of Washington
Seattle, Washington 98195

Introduction

While it is generally believed that iron requirements increase significantly during pregnancy, the extent of this increase is still the subject of considerable debate. Clearly an adequate hemoglobin level must be maintained throughout pregnancy to optimize maternal and fetal well-being. Without sufficient hemoglobin, oxygen transport to maternal and fetal tissues is compromised. While production of red blood cells is known to require a number of specific nutrients, iron is one of those most limiting in the American diet. Iron deficiency is one of the most common specific nutrient deficiencies seen in North America as well as in many other parts of the world.

Hematologic Alterations during Pregnancy

A fifty percent expansion in plasma volume begins early in the first trimester, peaks at 32 to 34 weeks of gestation in normal pregnancy and then plateaus until term. Over this same period of time, red cell volume rises linearly by about 20 to 30 percent. This phenomenon naturally leads to hemodilution such that hemoglobin and hematocrit values bottom out at 32 to 34 weeks of gestation and increase 6 to 8% by 40 weeks. Because of these physiologic adjustments, all laboratory blood indices must be compared by weeks of gestation rather than by trimester of pregnancy (1).

Assessment of hematological status during pregnancy may also require that race be taken into account. Garn et al (2) examined hematocrit and hemoglobin levels in 17,825 pregnancy examinations in the Collaborative Perinatal Project sponsored by the National Institutes of Health (3). The hemoglobin and hematocrit levels were, in each case, the lowest recorded for each woman studied during pregnancy. The income-corrected black/white difference in pregnant women was about 0.9 g/dl for hemoglobin and close to 2.6% for the hematocrit. Garn and colleagues (2) suggest that race-specific norms for these indices may be valuable. They point out that the net effect of race-specific hemoglobin and hematocrit norms would be to reduce the proportion of American blacks considered to be "anemic" or the proportion of black women needlessly given iron supplementation during pregnancy.

Iron Requirements during Pregnancy (Table 1)

The amount of elemental iron that the nonpregnant, nonlactating woman must transport from the gut to the circulation is only about 1.5 to 1.8 mg/day (1.0 mg to replace daily losses plus 0.5 to 0.8 mg/day to compensate for menstrual losses). During normal pregnancy, the iron requirement is 1.0, 2.0 and 4.0 mg/day to meet the needs, respectively, during the first, second and third trimesters (4). Obviously, the level of ingested iron must exceed by a substantial level these daily needs. A well-balanced American diet generally provides about 6.0 to 7.0 mg of iron per 1,000 kcalories or 12 to 14 mg of iron per day.

In fact, data from the Continuing Survey of Food Intakes by Individuals (1985), conducted by the United States Department of Agriculture, indicate that American women

of premenopausal age consume only about 82% of the RDA for energy and 61% of the RDA for iron (5). These patterns are maintained across races, socioeconomic groups and regions of the United States (Table 2). While there is evidence that iron absorption is enhanced to more than 20% during the latter half of pregnancy (6), this adaptation may not be sufficient to meet the proposed iron needs at this time.

Table 1. Iron "Cost" of a Normal Pregnancy

Iron contributed to the fetus	200-370 mg
Iron in placenta and cord	30-170 mg
Iron in blood lost at delivery	90-310 mg
Total	310-850 mg*

Data from: Bothwell TH, Charlton RW, Cook JD, and Finch CA: Iron Metabolism in Man. Oxford, Blackwell Pub. Co., 1979.

*These figures are in addition to the normal excretory loss of 0.5 to 1.0 mg/day and ignore the damand during the seocnd half of pregnancy for iron to support the expansion of red blood cell mass. This latter amount (200 to 600 mg) is not included as an iron "cost" because it is largely conserved (and not lost from the body) when the red blood cell mass returns to normal after delivery.

Parenthetically, with regard to iron absorption, one investigation (7) of changing levels of iron absorption during pregnancy was carried out under rather well-controlled circumstances. Labeled iron (in an ascorbic acid solution) was provided to fasting pregnant women at selected intervals during pregnancy. Iron absorption was carefully measured at each interval. The results indicated that iron absorption gradually improved as pregnancy proceeded, reaching a level well over 50% by term. However, this kind of study does not describe iron absorption as it normally occurs from mixed meals containing various levels of enhancers and inhibitors of iron absorption. The true efficiency of iron absorption in normal pregnant women is still uncertain.

The adverse impact on iron status of specific constituents in the diets of pregnant women continues to be of interest. Munoz et al (8) evaluated the impact of coffee consumption on hematological indices and trace element status of pregnant women and their infants in Costa Rica. Coffee drinkers (> 450 ml/day, n=22) and coffee nondrinkers (0 ml/day, n=26) were similar in income, education, prenatal care, age, parity, weight, height, pregnancy weight gain, prenatal iron supplementation, energy, protein, iron and vitamin C intake, and infant sex and gestational age. Maternal hemoglobin and hematocrit at 8 months gestation, cord blood hemoglobin and hematocrit, infant birthweight and hemoglobin and hematocrit at 1 month of age, and breast-milk iron concentration were significantly lower in the coffee group than in the non-coffee group. Interestingly, these results are similar to those reported previously in rats (9) and may indicate that maternal coffee intake contributes to maternal and infant anemia.

Maternal Iron Stores

If the diet of the pregnant woman does not satisfy her iron needs (even with improved iron absorption), it is then possible to draw upon available iron reserves to prevent the development of anemia. Under some circumstances, iron stores may be abundant and more than cover the extra iron demands of pregnancy. However, data available on the iron status of American women (as well as women in many other parts of the developing and developed world) suggest that at the very least, one in ten women maintains low iron reserves (10). Such women entering pregnancy would therefore be at high risk for the development of iron deficiency anemia unless food choices are markedly altered or iron supplementation is implemented.

Serum ferritin is generally regarded as a useful index of the status of iron stores (11-15). Several investigators have evaluated the changes in serum ferritin concentrations dur-

ing pregnancy and compared these changes with other usual measures of iron status. One such study involved healthy primigravidas who were not provided iron supplements. Serum ferritin concentration and hemoglobin, serum iron and transferrin levels were measured simultaneously. In first trimester pregnant women, serum iron and ferritin levels increased significantly as compared with those in nonpregnant women (14). During the second and third trimesters, serum iron and ferritin levels decreased significantly (Figure 1). However, hemoglobin levels showed little change and transferrin levels increased. These results suggest that maternal body iron storage is depleted during the second trimester in patients who are not receiving supplemental iron.

Table 2. Mean Intakes of Energy and Iron as Percentages of the 1980 Recommended Dietary Allowances: Women, one Day, spring, 1985, by Income, Race, Region and Urbanization

Population	Food Energy 82% (1660 kcals)	Iron 61% (11 mg)
All		
Income, as percentage of poverty threshold:		
- under 131%	73	62
- 131-300%	81	62
- over 300%	86	61
Race	82	61
White	82	61
Black	81	64
Region		
Northeast	79	64
Midwest	85	62
South	82	61
West	81	59
Urbanization		
Central City	83	63
Suburban	82	61
Nonmetropolitan	80	61

Source: Continuing Survey of Food Intakes of Individuals, 1985. USDA, Human Nutrition Information Service, Hyattsville, MD 20782.

Similar findings have been reported more recently by other investigators interested in comparing the progressive changes in iron status of pregnant women using iron supplements and similar women not using supplemental iron (12,13,15). Romslo et al (12) evaluated serum iron, iron binding capacity, serum ferritin and erythrocyte protoporphyrin in 45 healthy pregnant women; 22 were given oral iron while the others were given a placebo. When iron was not given, 15 out of 23 women had exhausted iron stores and iron deficiency at term, as judged from low serum ferritin, low serum transferrin saturation and high erythrocyte protoporphyrin values (Figure 2). Only 7 of them had a hemoglobin concentration between 10 and 11 g/dl at term but none had values <10 g/dl. In the iron treated group (n=22), none of the women developed iron deficiency. Serum ferritin was the most sensitive and specific test of iron deficiency.

In a similar study, Dawson and McGanity (15) selected 42 healthy young women, <16 weeks pregnant, with normal hematologic status. Each woman was randomly assigned to either a multivitamin/multimineral with 65 mg iron or one without iron. Those receiving the multivitamin without iron had

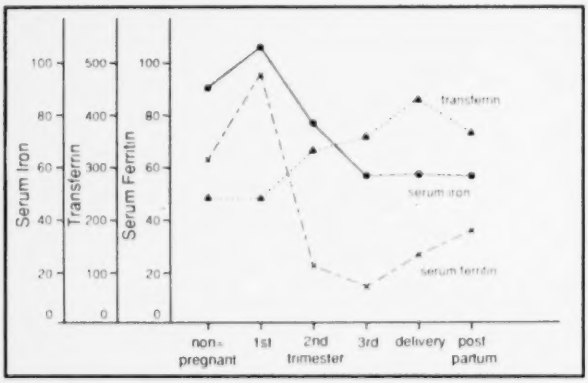

Figure 1. Hematologic changes during pregnancy. From: Kanesgige E: Serum ferritin as an assessment of iron stores and other hematologic parameters during pregnancy. Obstet. Gynecol. 57:238-242, 1981

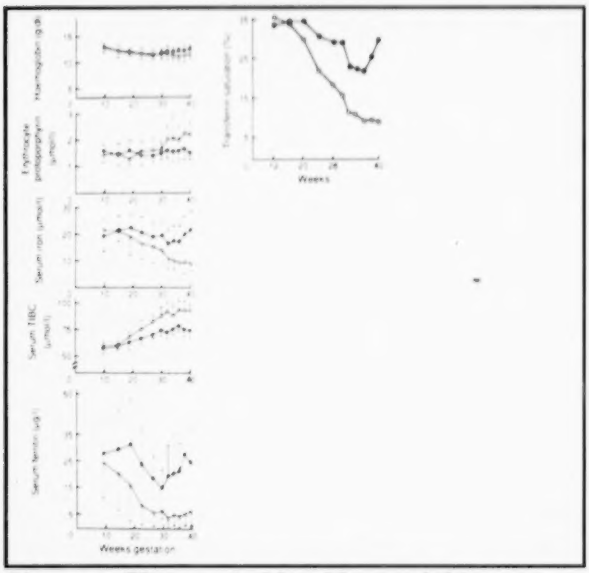

Figure 2. Changes in blood hemoglobin, erythrocyte protoporphyrin, serum iron, serum iron-binding capacity (TIBC), serum ferritin and transferrin saturation during pregnancy of women treated with (O) or without (Ō) oral iron supplementation. Results are given as mean values with the exception of transferrin saturation where +SD is omitted. From: Romslo I, Haram K, Sagen N, and Augensen K: Iron requirement in normal pregnancy as assessed by serum ferritin, serum transferrin saturation and erythrocyte protoporphyrin determinations. Br. J. Obstet. Gynaecol. 90:101-107, 1983

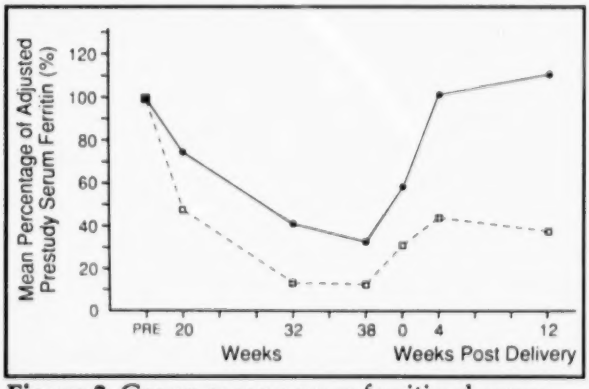

Figure 3. Group mean serum ferritin changes from prestudy baseline of adjusted control and supplement groups. O = supplement (n=21); += control (n=21). From Dawson EB and McGanity WJ: Protection of maternal iron stores in pregnancy. J. Repro. Med. 32:478-487, 1987

significantly lower mean serum ferritin levels (Figure 3). During the study, 9 of the 21 non-iron subjects (43%) failed to maintain an acceptable hemoglobin level (>11 g/dl) and were medicated with 110 mg ferrous iron daily. The researchers concluded that maternal iron stores cannot be maintained during pregnancy without the use of a low-level iron supplement.

If depletion of iron reserves occurs during pregnancy and iron supplements are not utilized, repletion of iron stores may require a considerable period of time. Taylor et al (16) measured serum ferritin levels in a population of 154 women of reproductive age. Serum ferritin concentrations ranged from 4.7 to 169 ug/1. Recent childbirth was associated with low serum ferritin values. Among those women not using iron supplements, it appeared to take up to two years after pregnancy before pre-pregnancy serum ferritin values were regained (Figure 4).

Romslo et al (12) have suggested that optimal prenatal care should include rational administration of iron supplements when a need is defined. They suggest that in otherwise healthy women up to 20 weeks gestation and with hemoglobin concentrations >11 g/dl, there is no need for iron supplementation or laboratory investigations other than hemoglobin estimations. At 20-24 weeks gestation, serum ferritin should be assessed since a low value signals exhausted iron stores and the need to consider iron supplementation. As pregnancy advances, body iron stores should be determined at intervals of 6 to 8 weeks by serum ferritin estimations and iron administered as required.

It is unfortunate that measurement of serum ferritin concentrations is not a simple and inexpensive office procedure. Because this is the case, effort is being made to find another laboratory test which adequately estimates iron stores but can be routinely handled by office nursing personnel. A promising candidate is the zinc protoporphyrin/ heme ratio, currently under study at the University of Washington in Seattle (17).

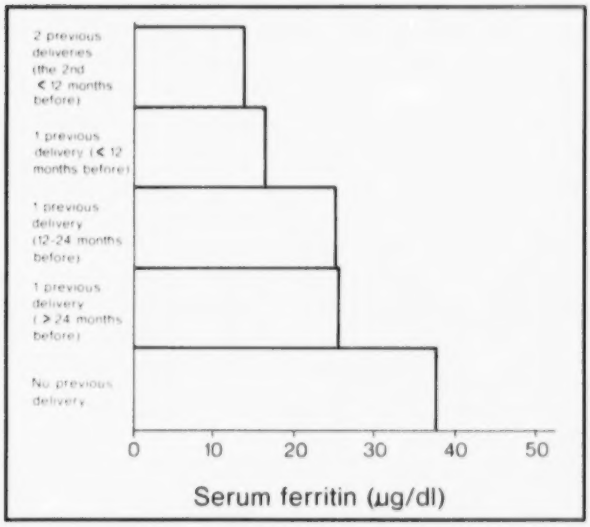

Figure 4. Effect of childbearing and period since last delivery on serum ferritin concentration. Based on data from: Taylor DJ, Mallen C, McDougall N, and Lind T: Serum ferritin in women of reproductive age. Br. J. Obstet. Gynaecol. 89:1000-1005, 1982

Routine Prenatal Iron Supplementation

The National Research Council currently recommends that pregnant women receive an oral iron supplement of 30 to 60 mg/day (18). Most prenatal vitamin/mineral supplements supply this recommended level of iron in the form of ferrous salts. While it is widely accepted that this supplemental iron will help maintain maternal iron stores, other benefits (if any) to the course and/or outcome of pregnancy are unclear.

Early Reviews. In 1982, Hemminki (19) discussed 20 clinical trials of vitamin and/or mineral supplements dating from 1936 which met minimal research design criteria. All of these studies were conducted on reasonably healthy pregnant women in developed countries. Little evidence was found of beneficial effects associated with routine vitamin, iron or other mineral supplements. The only study with a reasonable design showed no effect on pregnancy outcome (19,20).

Earlier studies found that women with low hemoglobins or who did not take iron and/or vitamins during pregnancy had lower birthweights or delivered prematurely. These studies, however, are no longer referenced because it is now recognized that variations in iron and vitamin levels reflect more fundamental problems or low socioeconomic status and poor general nutrition. Many of these studies were conducted during the 1940's through the 1960's, did not evaluate the women's total nutritional status (specifically kcalories and protein) and did not collect information on maternal prepregnancy weight or weight gain during pregnancy.

Iron Status of the Neonate. Maternal iron supplementation may benefit the infant. Efforts to determine whether or not the iron reserves of the offspring are affected by maternal iron status have generally shown a positive association (21-26). Singla et al (22) measured maternal plasma iron as well as storage iron in the fetal liver of 108 fetuses. A direct relationship was found between the concentration as well as the total content of storage iron in the fetal liver with the maternal plasma iron levels. These researchers suggest that babies born to iron deficient mothers have poor iron stores in their livers. Lubin et al (21) studied the effect of maternal iron status on the subsequent development of iron deficiency in the infant. Criteria for maternal iron deficiency were serum iron <42 mg/dl and % saturation <10%. These workers found that at 6 months of age, 70% of the infants of iron deficient mothers were iron deficient (% saturation 15%) compared to only 25% of infants of iron sufficient mothers. Maintenance of maternal iron reserves through low-level iron supplementation may therefore improve the iron status of the offspring.

Unnecessary Iron Supplementation. Excessive iron supplementation should be avoided for several reasons. First, supplemental iron is known to be associated with gastrointestinal discomfort in a number of individuals. Second, iron is known to inhibit zinc absorption if consumed at a ratio higher than 2:1. In a Colorado study (27), women taking daily supplements of 150 mg or more of iron had plasma zinc levels significantly lower

than those of women taking smaller amounts of iron. A third reason for avoiding excessive iron supplementation is that a small number of pregnant women may develop iron-induced macrocytosis (28), which could be taken as evidence of iron over-sufficiency. Animal studies have shown that macrocytes produced by increasing erythropoietin production and iron therapy have abnormal characteristics in that they have a reduced survival time and undergo intravascular fragmentation (29,30).

The Significance of Low or High Hemoglobin or Hematocrit Levels during Pregnancy.

While anemia is generally considered to be a threat to the quality of a pregnancy, controversy still exists about the significance of both low and high hematocrit and hemoglobin levels during pregnancy. Garn et al (31) explored maternal hematocrit and hemoglobin levels during pregnancy with respect to pregnancy outcomes in the 59,391 pregnancies included in the Collaborative Perinatal Project (3). Hematological data included the lowest hemoglobin and hematocrit encountered during pregnancy. Pregnancy outcomes included fetal death, perinatal death, gestational prematurity, low birth weight, low 1 and 5 minute Apgar scores and medical abnormality. A pregnancy resulting in a live term neonate in excess of 2500 grams with an acceptable Apgar score and lacking medical abnormality was defined as a "favorable" pregnancy outcome. As shown in Tables 3 & 4, there was an increased incidence of fetal deaths, short gestation lengths, low birth weights and medical abnormalities at both ends of the hematological distributions; undesirable outcome data for blacks are illustrated in Figure 5. Favorable outcomes for both whites and blacks (analyzed separately) were most likely at hemoglobin levels of approximately 11 to 12 g/dl and hematocrit levels of approximately 33 to 35% (). These findings were unchanged in trend after correction for maternal smoking, maternal obesity or socioeconomic status.

In a more recent study, Murphy et al (32) evaluated the relationship between hemoglobin concentrations at the first prenatal visit and subsequent outcome in 54,382 singleton pregnancies. Both high (>13.2 g/dl) and low (<10.4 g/dl) hemoglobin values were associated with an adverse outcome. Mothers with hemoglobin values in the intermediate range (10.4 - 13.2 g/dl) fared best. Significant differences emerged in perinatal mortality between those with high and those with intermediate hemoglobin levels at 13 to 19 weeks gestation. The frequencies of perinatal death,

Table 3

Percentage unfavorable outcomes by maternal pregnancy Hgb

n	Unfavorable pregnancy outcome	Hgb midpoint (g) 8	9	10	11	12	13	14
Whites								
3849	Medical abnormality	34.4	33.4	30.5	25.6	22.7	22.8	25.3
633	Fetal death	5.0	2.4	2.2	2.4	4.1	6.7	13.2
1677	Low birth weight*	13.8	11.5	9.7	8.9	9.0	11.4	11.0
2899	Prematurity†	21.5	19.8	17.6	15.0	14.7	18.6	22.3
Blacks								
4234	Medical abnormality	22.0	24.2	23.7	23.7	24.1	25.9	27.6
563	Fetal death	2.6	1.6	2.0	2.7	5.4	9.7	14.5
3134	Low birth weight	15.9	14.9	15.5	15.9	17.9	22.4	30.4
5611	Prematurity	30.2	28.1	28.4	27.5	28.9	32.5	40.8

* <2500 g.
† <37 wk.
From: Garn SM, Keating MT, and Falkner F: Hematological status and pregnancy outcomes. Am. J. Clin. Nutr. 34:115-117, 1981.

Table 4

Percentage unfavorable outcomes by maternal pregnancy Hct

Unfavorable pregnancy outcome	Maternal Hct midpoint 23	25	27	29	31	33	35	37	39	41	43
Whites											
Medical abnormality	43.2	34.5	30.9	33.8	29.0	25.6	22.6	22.4	22.2	20.0	22.0
Prematurity	25.5	30.2	23.4	17.3	16.7	14.4	13.8	15.3	17.9	19.8	28.6
Low birth weight	15.4	20.5	13.4	9.5	10.2	8.4	8.2	9.2	11.4	9.7	15.9
Fetal death	11.3	6.9	5.4	2.5	2.3	2.3	2.7	3.7	5.8	9.8	18.5
Blacks											
Medical abnormality	33.3	28.8	29.0	26.0	25.9	24.7	25.6	27.1	28.0	26.5	31.0
Prematurity	38.4	33.7	30.9	27.1	29.4	26.9	27.8	30.2	34.2	37.1	37.1
Low birth weight	21.1	21.2	17.5	15.7	15.3	15.4	16.1	19.5	21.6	22.9	33.0
Fetal death	5.9	4.3	2.5	2.0	2.0	2.5	3.3	4.7	8.7	16.0	16.1

From: Garn SM, Keating MT, and Falkner F: Hematological status and pregnancy outcomes. Am. J. Clin. Nutr. 34:115-117, 1981.

low birthweight, and preterm delivery were greater with high than with intermediate hemoglobins. There was also a striking relation between first-visit hemoglobin values and the subsequent frequency of hypertension (Figure 7). In primiparas, the frequency of subsequent hypertension ranged from 7% at hemoglobin values under 10.5 g/dl to 42% at hemoglobin concentrations over 14.5 g/dl. The authors suggest that the adverse findings among the high hemoglobin group may reflect some failure in plasma volume expansion in these women. This could, in turn, have a deleterious effect on the uteroplacental circulation. Other researchers have similarly found that high hemoglobin/hematocrit is associated with adverse pregnancy outcome (33-36).

Lieberman et al (37) reviewed data from 12,718 singleton pregnancies which were monitored at the Boston Hospital for Women from August 8, 1977, to March 31, 1980. An increasing rate of prematurity was found to be associated with decreasing maternal hematocrit (Figure 8). The lowest risk of premature birth was found when maternal hematocrit was between 41% and 44%. Statistically significant increases in prematurity were noted at all hematocrit levels of <38%. The authors suggest that a number of mechanisms could account for why low hematocrit is a risk factor for adverse pregnancy outcome. It might be a marker of some other risk such as poor nutrition, infection or some other as yet unknown factor. Such a factor, they hypothesize, could independently cause both low hematocrit and premature labor. Alternatively, they propose, low hematocrit could lead directly to

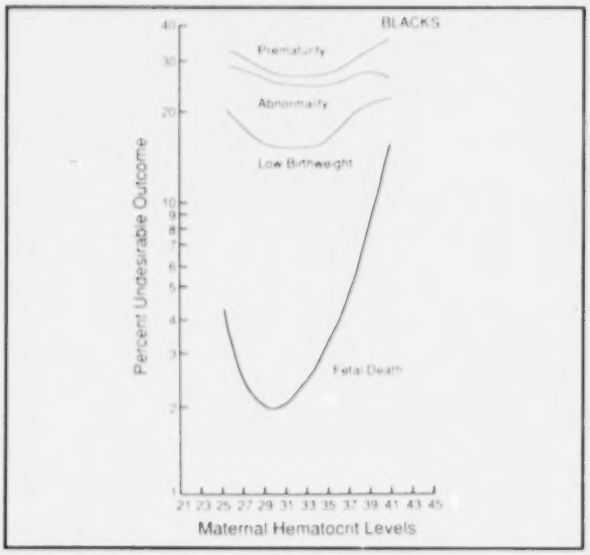

Figure 5. Relationships between lowest maternal hematocrit during pregnancy and four unfavorable outcomes (fetal death, low birth weight, prematurity and medical abnormality). From: Garn SM, Keating MT, and Falkner F: Hematological status and pregnancy outcomes. Am. J. Clin. Nutr. 34:115-117, 1981

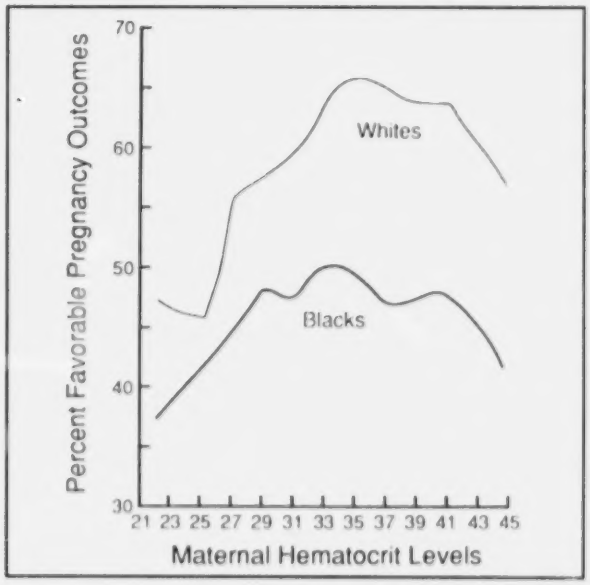

Figure 6. Relationship between lowest maternal hematocrit levels during pregnancy (abscissa) and percentage favorable pregnancy outcome (ordinate). As shown separately for blacks (solid line) and whites (dashed line) the highest percentage of favorable pregnancy outcome is 33 to 35% packed cell volume range, higher for whites and lower for blacks. Lowest maternal hemoglobin levels during pregnancy similarly show optima in the middle of the distribution approximating 11 g for blacks and 12 g for whites. From: Garn SM, Keating MT, and Falkner F: Hematological status and pregnancy outcomes. Am. J. Clin. Nutr. 34:115-117, 1981

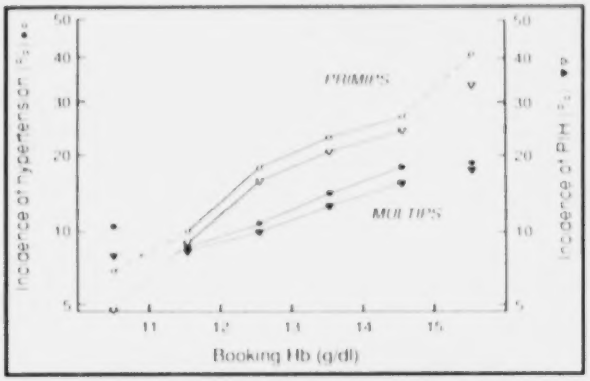

Figure 7. Frequency of all hypertension in pregnancy and pregnancy-induced hypertension among primiparas and multiparas according to hemoglobin concentration at first prenatal visit. (Points representing hemoglobin groups <10.5 g/dl and >14.5 g/dl have been joined to their neighbors by broken lines to indicate that these values may be prone to inaccuracy because of small numbers). From: Murphy JF, Newcombe RG, O'Riodan J, Coles EC, and Pearson JF: Relation of hemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet 1:992-995, 1986

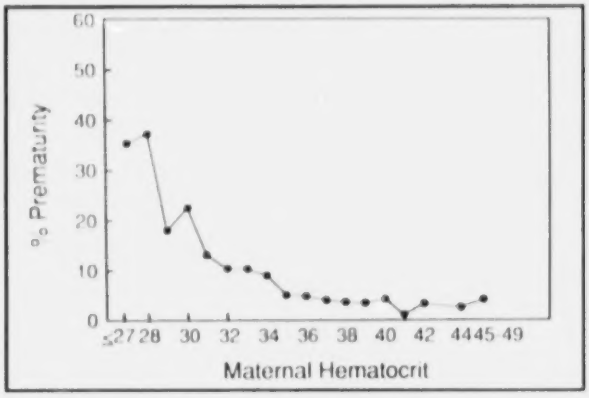

Figure 8. Percent spontaneous premature birth according to maternal hematocrit (women with hematocrit levels from 45% to 49% were combined because of small numbers). From: Lieberman E, Ryan KJ, Monson RR, and Schoenbaum SC: Association of maternal hematocrit with premature labor. Am. J. Obstet. Gynecol. 159:107-114, 1988

adverse pregnancy effects possibly as a result of decreased delivery of oxygen to the fetus. Lastly, they suggest, the possibility exists that an abnormal pregnancy itself might lead to both low hematocrit and premature birth.

Researchers from the Prevention Research Program at the National Institute of Child Health and Human Development have recently argued that maternal anemia is not associated with preterm delivery (38). Klebanoff et al examined prospectively the effect of anemia (hematocrit <0.34) on subsequent preterm birth in 35,423 pregnancies from the Collaborative Perinatal Project (1959-1966). The incidence of preterm birth among women with and without anemia at each week during the third trimester was compared. While a weak association was noted between anemia and preterm delivery early in the third trimester, this did not account for the substantial increase in preterm birth seen among black women. Anemia after 30 weeks gestation was not associated with preterm birth. When the hematocrits of women in term labor were compared with those of women in preterm labor, a spurious dose-response effect of anemia was created (Figure 9,10). The NICHD researchers reached the following conclusions: 1) Hematocrits rise throughout the third trimester in normal pregnancies, particularly among black women (Figure 11). 2) Because of this, studies comparing hematocrits obtained from women in term and preterm labor are likely to yield a spurious positive association between anemia and preterm delivery. 3) Anemia is not a strong risk factor for preterm birth when an appropriate comparison group is used. In view of these observations, this research group does not believe that there is sufficient evidence to justify a randomized clinical trial of treatment of borderline anemia during pregnancy.

Conclusion

Iron is a required nutrient for both mother and fetus. Assuring that maternal iron intake is satisfactory therefore is a justifiable goal in prenatal care. Dietary iron alone can satisfy the iron needs of some pregnant women but a

sizeable percentage of this population will draw heavily upon their iron stores to maintain normal hematological status. While reduced maternal iron reserves does not appear to be associated with adverse pregnancy outcome, one might suggest that such a condition at delivery is less than optimum for both mother and baby. Low-level iron supplementation may therefore be justifiable, especially during the latter half of pregnancy when evidence of reduced iron stores is available.

Treatment of iron deficiency anemia with an appropriate dosage of an iron supplement will certainly improve maternal iron status and probably will reduce the risk of adverse pregnancy outcome. It is clear that controversy still exists about the impact of anemia on risk of preterm birth but it would appear that Klebanoff and colleagues make a rather strong case for not investing in a randomized clinical trial of treatment of borderline anemia during pregnancy.

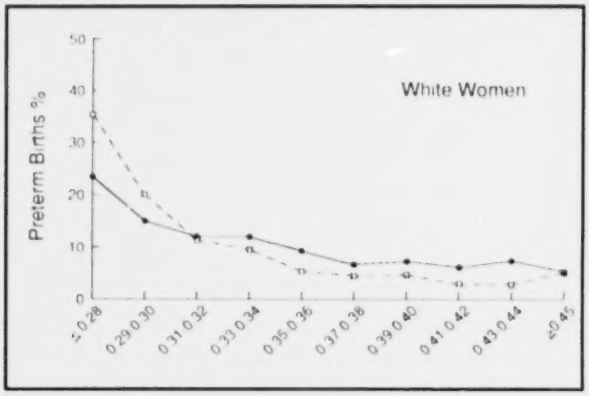

Figure 10. Artifactual relationship between hematocrit and the risk of preterm birth in white women. Solid line indicates data from the Klebanoff et al (38) study; broken line, data from Lieberman et al (37). From: Klebanoff MA, Shiono PH, Berendes HW, and Rhoads GG: Facts and artifacts about anemia and preterm delivery. J. Am. Med. Assoc. 262:511-515, 1989

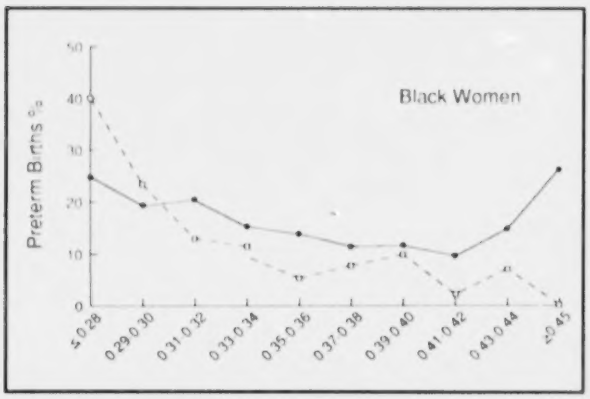

Figure 9. Artifactual relationship between hematocrit and the risk of preterm birth in black women. Solid line indicates data from the Klebanoff et al (38) study; broken line, data from Lieberman et al (37). From: Klebanoff MA, Shiono PH, Berendes HW, and Rhoads GG: Facts and artifacts about anemia and preterm delivery. J. Am. Med. Assoc. 262:511-515, 1989

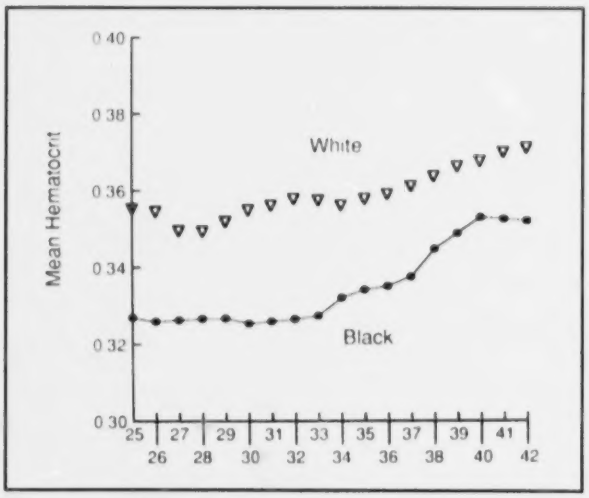

Figure 11. Mean hematocrit at each week of gestation among black and white women delivering term infants weighing 2500 g or more. From: Klebanoff MA, Shiono PH, Berendes HW, and Rhoads GG: Facts and artifacts about anemia and preterm delivery. J. Am. Med. Assoc. 262:511-515, 1989

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IRON DEFICIENCY: PEDIATRIC EPIDEMIOLOGY

Allan A. Johnson.
Department of Human Nutrition and Food,
School of Human Ecology, Howard University, Washington, DC

Introduction

Iron deficiency is recognized as the most common nutritional deficiency in both developed and less developed countries. It is particularly widespread among infants, young children, and adolescents.

Iron Needs During Infant and Child Development

During pregnancy, the fetus accumulates iron at a rate that is directly proportional to its increase in body weight (1). It is frequently stated that the fetus behaves as a highly effective parasite of maternal iron, drawing whatever iron it needs from the maternal circulation (2). However, the results of studies on the relationship between maternal and infant iron status are conflicting (3-8). It is possible that the conflicting results may be explained by differences in the criteria used to define iron status, and in the degree of iron deficiency in the mothers. Mild to moderate degrees of iron deficiency may have no impact on the iron status of newborn infants, while severe iron deficiency may significantly decrease infant iron status.

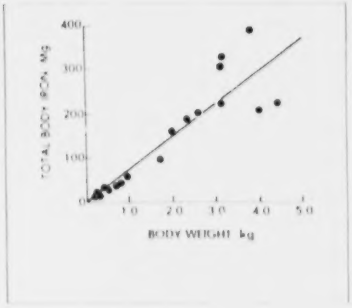
Under normal circumstances, the newborn infant is well supplied with iron. At term, total body iron averages 75 mg./kg., a level which is maintained throughout fetal life and is approximately twice that of the adult male. In low birth weight infants, total body iron is less than that in full-term infants, but the ratio of iron to body weight is the same (8).

Figure 1 illustrates the linear relationship between total body iron and infant birth weight.

After birth, there are considerable changes in iron metabolism, particularly in iron balance and erythropoiesis.

Figure 1

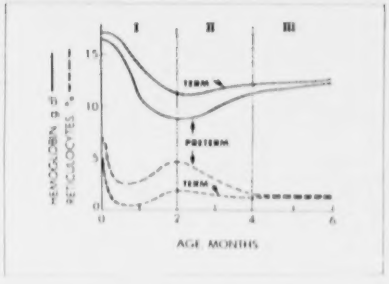
TOTAL BODY IRON VS INFANT BIRTH WEIGHT



SOURCE: ARCH DIS CHILD 1951; 26:205-214

Figure 2

POSTNATAL STAGES OF IRON BALANCE AND ERYTHROPOIESIS



SOURCE: INACG 1979; WASH DC: NUTRITION FOUNDATION

Postnatal iron balance and erythropoiesis can be conveniently divided into three stages, (Figure 2), (8). The first stage occurs between birth and the first eight weeks of life. It is characterized by a marked decline in erythropoiesis in response to increased

postnatal delivery of oxygen to the tissues. In the bone marrow, the percentage of erythroid precursors falls from 30% at birth to 10% by one month of age and extramedullary hematopoiesis ceases. In the peripheral blood, the hemoglobin level falls from an average of 17.0 g./dl. at birth to 11 g./dl at eight weeks, the lowest hemoglobin level during any stage of development. Also, the reticulocyte count falls precipitously. Most of the iron released by the destruction of senescent red cells is stored, as only a small proportion is utilized for erythropoiesis. As a result of the increase in iron stores, the percentage of dietary iron absorbed declines. However, there is little need for exogenous iron during this stage (9).

During the second stage, between two and four months, there is an increase in erythropoiesis, leading to an increase in the percentage of erythroid precursors in the bone marrow. Consequently, the hemoglobin level rises from 11 g/dl to an average to 12.5 g/dl. during the remainder of the first year of life. The reticulocyte count rises to far above the normal adult level.

The third stage is characterized by a continued increase in erythropoiesis as a result of rapid growth rate and expansion in blood volume. The iron store decreases and the infant becomes dependent upon dietary iron. An average amount of 0.7mg. of iron/day must be absorbed to provide for growth, and 0.2 mg./day to balance basal losses (9). If the iron intake is inadequate, the stores will become exhausted, and iron deficiency will follow.

In preterm or low birth weight infants, there is a similar postnatal fall in hemoglobin to a low value between six weeks and two months of age. However, this low value (approximately 9 g/dl.) is less than in the term infant. The increase in erythropoiesis that occurs during the second stage is greater in preterm than in term infants.

Low birth weight infants have more rapid growth rates than term infants, and exhaust their iron stores earlier. Therefore, the requirements for exogenous iron are greater than in the term infant, and third stage of iron nutrition begins at an earlier age. If the supply and absorption of dietary iron are adequate, the hemoglobin level in preterm infants reaches that of term infants until mean values are equal by nine months of age (3).

Figure 3

CHANGES IN BODY IRON DURING INFANCY

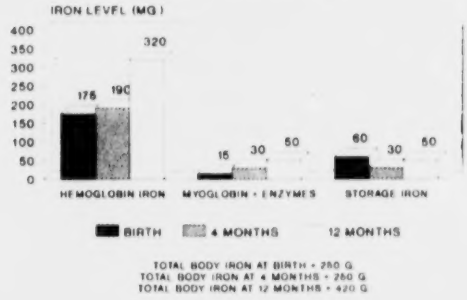


Figure 3 shows the changes in the various fractions of body iron during the first twelve months of life. Functional iron in hemoglobin, myoglobin and enzymes increases progressively during the first year of life (9). Storage iron is highest at birth, decreasing to its lowest level at 4 months which coincides with the beginning of the third stage of iron nutrition when the infant is heavily dependent on dietary iron. By twelve months, iron stores have increased to 50 mg.

During preadolescent childhood, there is a continuation of the dependence on dietary iron which began in late infancy. The main reason for this is the rapid growth rate and expansion of blood volume. Compared with infancy, however, the demand for iron during childhood is lower per kilogram of body weight because of the relatively lower growth rate.

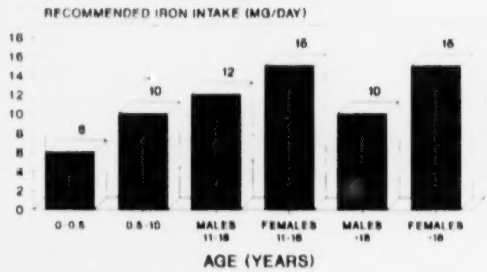
During the adolescent growth spurt, there is a marked increase in the demand for iron primarily for the production of hemoglobin

(8). For example, during the peak year of adolescent growth, boys gain an average of 10 kg. This requires a net increase of about 300 mg. of iron to maintain a constant hemoglobin concentration as the blood volume expands. An additional 50 mg. of iron is needed on the average to allow for the increase in hemoglobin concentration of 0.5-1.0 g/dl/year.

In adolescent girls, the increase in iron demand is not as rapid as in boys because the maximum yearly weight gain is somewhat less, and the hemoglobin concentration increases only slightly. The average weight gain of 9 kg/year in adolescent girls requires about 280 mg. of iron for the maintenance of a constant hemoglobin concentration. The onset of menses imposes an additional demand of 175 mg. of iron/year, based on a median menstrual loss of 30 ml. of blood per menstrual period.

Figure 4

RECOMMENDED DAILY INTAKES OF IRON



SOURCE: FOOD AND NUTRITION BOARD, NATIONAL RESEARCH COUNCIL. RECOMMENDED DIETARY ALLOWANCES, 10th ED. 1989

Figure 4 shows the U.S. Recommended Daily Allowances of iron for various age groups (10). Expressed per kilogram of body weight, recommended iron intakes of infants and children exceed those of adults.

Etiology of Iron Deficiency During Infancy and Childhood

Iron deficiency occurs when the amount of iron absorbed is insufficient to meet the needs of the body. There are four major determinants of iron status during infancy and childhood (Table 1). These include body iron content at birth, the rate of growth, iron intake and absorption, and blood losses (1,2).

Table 1

DETERMINANTS OF IRON STATUS DURING INFANCY AND CHILDHOOD

- BODY IRON CONTENT AT BIRTH
- RATE OF GROWTH
- IRON INTAKE AND ABSORPTION
- BLOOD LOSSES

It has previously been reported that the body iron content at birth is directly proportional to the birth weight. Therefore, low birth weight infants are highly susceptible to iron deficiency unless they are supplemented with iron. The normal term infant is born with sufficient body iron to sustain erythropoiesis for four to six months (11).

The single most important factor responsible for the high iron requirements of infants and children is their rapid growth rates. The highest prevalence of iron deficiency is likely to occur between about six months and two to three years when the iron stores may become depleted in the face of rapid growth. A second period of rapid growth occurs during adolescence (11). Therefore, the second peak incidence of iron deficiency is found among adolescents, primarily in adolescent girls because of the additional menstrual losses.

During the first few months of life, dietary iron is derived almost exclusively from milk. The iron content of both human and unfortified cow's milk is low (1). The iron content of breast milk falls gradually from about 0.5 mg/l. during the first month after birth, to about 0.3 mg/l. between 4 and 6 months after birth (8). The iron content of unfortified cow's milk averages 0.8 mg/l. In spite of the low iron content of breast milk, exclusive breastfeeding supplies the full-term infant

with enough iron to prevent iron deficiency for the first six months of life. Unfortified cow's milk and cow's milk formula, on the other hand, do not supply the infant with adequate iron to maintain optimal balance (1). The reason for the difference between the two types of milk rests with the relative rates of absorption of iron. An average of 49% of the iron in breast milk is absorbed, compared with 10-12% from unfortified cow's milk and cow's milk formula. Table 2 shows the factors responsible for the higher rate of absorption of iron from breast milk (8).

Table 2

FACTORS RESPONSIBLE FOR HIGHER ABSORPTION OF IRON IN BREAST MILK

- HIGHER CONCENTRATION OF LACTOFERRIN
- LOWER CONCENTRATION OF PROTEIN
- LOWER CONCENTRATION OF CALCIUM
- LOWER CONCENTRATION OF PHOSPHATE

SOURCE: INDIAN J PEDIATR 1987; 54: 323-342

Most iron-fortified cow's milk formulas contain between 11 and 12 mg. iron per liter as ferrous sulfate or ferrous gluconate. The average absorption of iron from fortified formulas is about 4% (8). Although the percentage absorption is less than half that of iron in unfortified formula, the amount of iron retained is more than six times greater.

By 4-6 months of age, solid foods are progressively introduced into the infant's diet. The ratio of heme to non-heme iron in the diet, and several other dietary factors influence the bioavailability of iron in weaning foods.

Heme iron present in hemoglobin and myoglobin of animal tissues is well absorbed regardless of the composition of the diet (1). Non-heme iron present in vegetables, cereals and in some foods of animal origin, such as milk and eggs, is generally poorly absorbed, the rate of absorption being greatly influenced by several factors. Table 3 shows the factors responsible for enhanced non-heme iron absorption (1,2). Dietary ferric iron is converted to the more soluble ferrous form by hydrochloric acid present in gastric juice, and by ascorbic acid. Several organic acids - ascorbic, lactic, citric and malic - promote absorption of non-heme iron by forming soluble iron complexes. Ascorbic acid is the most potent of these, however. Amino acids such as histidine and lysine, and sugars (lactose and fructose) also form soluble complexes with iron and, therefore, promote its absorption. The lower the body stores of iron, the greater its rate of absorption from the diet. The presence of meat enhances the absorption of non-heme iron in a mixed meal.

Table 3

FACTORS PROMOTING ABSORPTION OF NON-HEME IRON

- HYDROCHLORIC ACID
- ORGANIC ACIDS - ASCORBIC ACID, LACTIC ACID CITRIC ACID, MALIC ACID
- AMINO ACIDS - LYSINE, HISTIDINE
- SUGARS - FRUCTOSE, LACTOSE
- LOW IRON STORES
- MEAT

Several factors inhibit the absorption of non-heme iron. All of the substances listed in Table 4 inhibit non-heme iron absorption

Table 4

FACTORS INHIBITING ABSORPTION OF NON-HEME IRON

- PHYTATE
- PHOSPHATE
- OXALATE
- POLYPHENOLS
- TANNINS
- FIBER
- EGG YOLK PHOSPHOPROTEIN
- EDTA

by forming insoluble complexes with it.

Blood loss is not a major cause of iron deficiency in the United States. However, it must be considered whenever iron deficiency fails to respond to therapeutic iron. Sensitivity to a heat labile protein in cow's milk may cause occult gastrointestinal bleeding. This occurs frequently in infants fed whole cow's milk at an early age (before 6 months) in amounts exceeding 1 quart/day. Blood losses as high as 10 ml./day have been documented in infants with this syndrome (11). Use of heat processed formula usually reverses this blood loss, presumably due to the denaturation of the protein allergen believed to be lactalbumin (9. In addition, there are several medical causes of gastrointestinal bleeding in children, which are beyond the scope of this article.

Table 5

PREVENTION OF IRON DEFICIENCY USING UNFORTIFIED FOODS

1. BREASTFEED INFANTS FOR AT LEAST 6 MONTHS
2. DO NOT FEED FRESH COWS MILK TO INFANTS UNTIL AFTER THEY ARE 9 MONTHS OF AGE
3. FEED INFANTS AND CHILDREN ASCORBIC ACID-RICH FOODS AND FRUIT JUICES AND/OR MEAT WITH MEALS OF SOLID FOODS AFTER ABOUT 6 MONTHS

SOURCE: ACTA PAEDIATR SCAND SUPPL 1986; 323: 59-67

Prevention of Iron Deficiency

Table 5 shows approaches that can be used for the prevention of iron deficiency in infants and children using unfortified foods (9). Infants who are breast-fed initially, and then given the appropriate solid foods can avoid iron deficiency without iron-fortified foods. It has previously been stated that exclusive breast-feeding provides infants with sufficient iron to maintain adequate iron status for the first six months of life. Because of the possibility of occult bleeding, fresh cow's milk should not be fed to infants until after 9 months of age. The American Academy of Pediatrics has recommended the introduction of fresh cow's milk as early as six months of age, so that it can be used earlier than 9 months if this is desirable.

After the introduction of solid foods, iron deficiency can be prevented in infants and children by making use of appropriate food combinations. The consumption of ascorbic acid-rich foods and/or meat together with plant foods leads to enhanced absorption of non-heme iron in these plant foods. For example, enriched cereals and legumes are rich in iron but the iron is rather poorly absorbed when these foods are eaten alone. The addition of relatively small amounts of meat, fish, or poultry will result in a marked increase in the absorption of iron from the entire meal. Alternatively, ascorbic-rich juices such as orange juice, or ascorbic acid-rich vegetables such as cauliflower can produce the same effect at much lower cost (9).

Table 6

PREVENTION OF IRON DEFICIENCY USING FORTIFIED FOODS

1. FEED INFANTS FORMULA FORTIFIED WITH IRON AND ASCORBIC ACID
2. FEED INFANTS CEREAL OR MILK-CEREAL PRODUCTS THAT ARE FORTIFIED WITH IRON, OR IRON AND ASCORBIC ACID
3. USE ASCORBIC ACID-FORTIFIED FRUIT JUICES WITH MEALS OF SOLID FOODS

SOURCE: ACTA PAEDIATR SCAND SUPPL 1986; 323:59-67

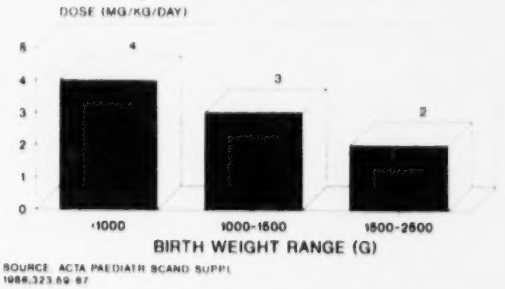
Table 6 shows the approaches that can be used for the prevention of iron deficiency in infants and children using fortified foods (9). Iron and ascorbic acid-fortified formula constitute an appropriate substitute for breast milk during the first six months of life, and are the preferred method of milk feeding between 6 and 12 months of age. Studies in Chile showed that the addition of 100 mg. of ascorbic acid per liter caused a doubling in

the absorption of iron from a formula fortified with 15mg of iron (as ferrous sulfate) per liter (9). This indicates that the addition of ascorbic acid largely accounts for the effectiveness of iron-fortified cow's milk formula in preventing iron deficiency.

Iron-fortified cereals are recommended as the first solid foods for infants. The bioavailability of fortification iron has been an important issue with respect to infant cereals (9). Prior to 1972, infant cereals in the United States were fortified with phosphate compounds which proved to be virtually unabsorbable. Currently, infant cereals are fortified with reduced or electrolytic iron which has a relatively small particle size and a large reactive surface area that would favor a high bioavailability (8). The additional fortification of iron-fortified cereals with ascorbic acid should result in the increased absorption of iron from such cereals. In the absence of such fortification with ascorbic acid, the iron-fortified cereals can be consumed together with ascorbic acid-rich fruits and juices, or juices fortified with ascorbic acid.

Figure 5

RECOMMENDED DOSES FOR IRON SUPPLEMENTATION OF PRETERM INFANTS



SOURCE: ACTA PAEDIATR SCAND SUPPL 1986;323:69-87

In preterm infants, especially in those who are being breast fed exclusively, iron supplementation is necessary for the prevention of iron deficiency. The dose varies according to birth weight (Figure 5). Prevention of iron deficiency can be achieved by the provision of 4 mg. iron/kg. body weight/day in infants with birth weights below 1000 g.; 3 mg./kg body weight/day in infants with birth weights between 1000 and 1500 g.; and 2 mg./kg. body weight/day in infants with birth weights between 1500 and 2500 g. Prior to one year of age, iron supplements are provided in the liquid form as ferrous sulfate or a similarly bioavailable compound such as fumarate or gluconate. Subsequently, chewable tablets can be used. Iron supplementation of preterm infants should be given between 2 and 8 weeks of age and should be continued for at least the first 12 months of life. The supplemental iron should be given between meals or at least 30 minutes before a meal (9).

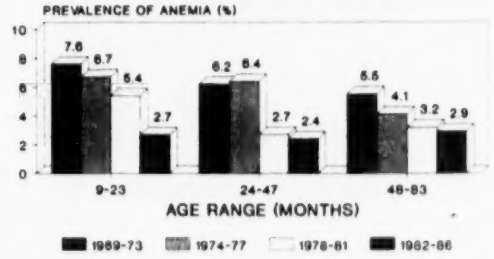
It must be mentioned that, compared with dietary modification, iron supplementation is a definite second choice for the prevention of iron deficiency. The major problem is compliance. For this reason, dietary modification and/or the use of iron-fortified foods are preferred to supplementation for the prevention of iron deficiency in infants and children, except for breast-fed preterm infants and other infants and children with iron deficiency anemia. One other group which may require iron supplementation is adolescent girls who have relatively high iron requirements which in many cases cannot be easily met by dietary means.

Trends in the Prevalence of Iron Deficiency Among Infants and Children in the United States

There have been several reports of a declining prevalence of anemia among children in the United States (9, 11-15). Two of these reports have been selected for presentation here. Yip et al. (14) conducted a 17-year retrospective chart review of hematocrit measurements of middle-class children. The 1,682 children, aged between 9 and 83 months, were seen at a private pediatric clinic in Minneapolis from September 1969 through May 1986, and were predominantly white. Age-specific values for hematocrit were used to define anemia (Figure 6). The authors reported a consistent and statistically significant decline in the prevalence of ane-

Figure 6

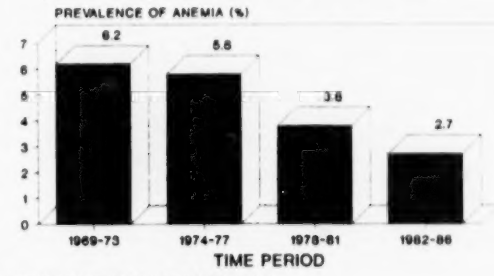
PREVALENCES OF ANEMIA* BY AGE AND TIME PERIOD



*CHILDREN<2 YR-HCT<33%; CHILDREN 2-5 YR-HCT<34%; CHILDREN 6-7 YR-HCT<35%
SOURCE: PEDIATRICS 1987; 80: 330-334

Figure 7

OVERALL PREVALENCES OF ANEMIA* BY TIME PERIOD



*CHILDREN<2 YR-HCT<33%; CHILDREN 2-5 YR-HCT<34%; CHILDREN 6-7 YR-HCT<35%
SOURCE: PEDIATRICS 1987; 80: 330-334

mia among healthy children for three age-groups during the four time periods studied. After adjustment for age, the overall prevalence of anemia declined from 6.2% during 1969-1973, to 2.7% between 1982 and 1986 (Figure 7). The authors attributed the decline in the prevalence of anemia to changes in feeding practices during the past 20 years (Table 7). These included the substitution of iron-fortified formulas for unfortified formulas and cow's milk, more prolonged use of fortified formulas during the first year of life, a greater frequency and duration of breast-feeding, and the increased use of iron-fortified infant cereals. Further, results of the 1985 National Food Consumption Survey showed a higher average daily intake of iron among children aged 1-5 years, compared

Table 7

SUGGESTED CHANGES IN FEEDING PRACTICES

- SUBSTITUTION OF IRON-FORTIFIED FORMULAS FOR UNFORTIFIED FORMULAS AND COW'S MILK
- MORE PROLONGED USE OF FORTIFIED FORMULAS DURING THE FIRST YEAR OF LIFE
- GREATER FREQUENCY AND DURATION OF BREAST FEEDING
- INCREASED USE OF IRON-FORTIFIED INFANT CEREALS

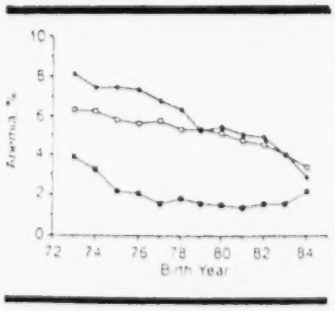
SOURCE: JAMA 1987; 258: 1619-1623

with the 1977 survey.

Yip et al. (15) conducted an anemia trend analysis of hematologic measurements ob-

Figure 8

RACE-SPECIFIC PREVALENCES OF ANEMIA BY BIRTH-YEAR COHORT



SOURCE: JAMA 1987; 258: 1619-1623

—Race-specific pervalence of anemia based on birth-year cohort, six selected states in Centers for Disease Control Pediatric Nutrition Surveillance System. Top line indicates black children; middle line, white children; and bottom line, American Indian children.

tained from low-income children aged between 6 and 60 months who were enrolled in public health programs in six states. The public health programs in the six states - Arizona, Kentucky, Louisiana, Montana, Oregon and Tennessee - were continuously monitored by the Centers for Disease Control Pediatric Nutrition Surveillance System. A total of 1, 680, and 740 hemoglobin and hematocrit measurements respectively were obtained from 499,759 children. Age-specific

hemoglobin and hematocrit cutoff values were used to define anemia (Figure 8).

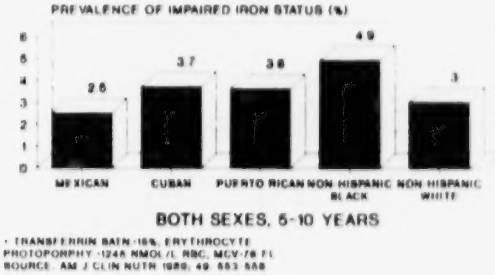
Race specific prevalence of anemia by birth-year cohort for white, African American, and American Indian children are shown. For all races, the prevalence of anemia declined significantly. Though African American children had a higher prevalence of anemia than white children in the earlier years, the gap between African Americans and whites narrowed as the prevalence of anemia declined. In each year, American Indian children from Arizona had a significantly lower rate of anemia than either African American or white children. This could be attributed to their participation in the Special Supplemental Food Program for Women, Infants and Children (WIC). The authors concluded that the decline in the prevalence of anemia probably resulted from improved iron status among these children. The general improvement in iron intake among U.S. infants and children, and the benefits of the WIC Program may have contributed to the improvement in iron status.

More recently Looker et al. (16) have published data on the prevalence of impaired iron status among three Hispanic groups and non-Hispanic blacks and whites in the United States. The Hispanic data were derived from the Hispanic Health and Nutrition Examination Survey (HHANES) conducted between 1982 and 1984. Data for the non-Hispanic blacks and whites came from the Second National Health and Nutrition Examination Survey (NHANES II) conducted between 1976 and 1980. HHANES was a probability survey of three special population subgroups aged between 6 months and 74 years in selected areas: Mexican-Americans from five southwestern states (Arizona, California, Colorado, New Mexico and Texas): Cuban-Americans from Dade County, Florida; and Puerto Rican Americans from the New York City Metropolitan Area. NHANES II was a national probability sample of the noninstitutionalized U.S. population ages 6 months through 74 years. The data to be presented here are based on impaired iron status as defined by the mean corpuscular volume (MCV) model. To be considered iron-impaired by the MCV model, an individual must have had abnormal values for at least two of the three iron status indicators (MCV), erythrocyte protoporphyrin, and transferrin saturation). Age-specific cutoff values were used to indicate abnormality for each of these indicators (Figure 9,10, 11). The MCV model was designed to indicate iron-deficient erythropoiesis.

Figure 9 shows relatively low prevalence of impairment of iron status among children

Figure 9

PREVALENCE OF IMPAIRED IRON STATUS* BY POPULATION GROUP

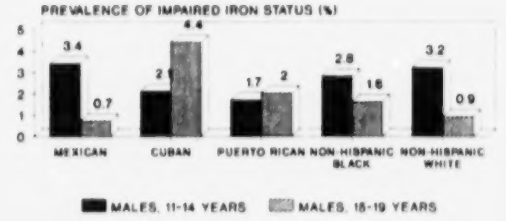


aged 5-10 years, in all the population groups studied. The prevalence was highest among non-Hispanic blacks, and lowest among Mexican-Americans. These findings appear to support the reports by Yip et al. (14, 15) on the low prevalence of iron deficiency among U.S. children.

Figure 10 shows that the prevalence of impaired iron status was low among the various population groups of males aged between 11 and 19 years. Among females at ages 11-19 years (Figure 11), the prevalence of impaired iron status was somewhat higher than that among similarly aged males. Menstrual blood losses may have contributed to this higher prevalence of iron impairment among females. As in children aged 5-10 years, the prevalence of impairment of iron

Figure 10

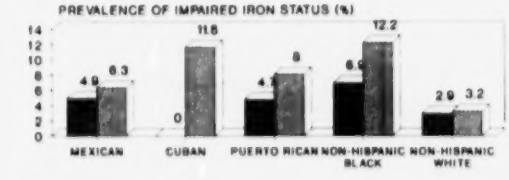
PREVALENCE OF IMPAIRED IRON STATUS* BY POPULATION GROUP



* TRANSFERRIN SATN. <16%, ERYTHROCYTE PROTOPORPHY. >124.6 NMOL/L RBC, MCV <78 FL
SOURCE: AM J CLIN NUTR 1989; 49: 553-558

Figure 11

PREVALENCE OF IMPAIRED IRON STATUS* BY POPULATION GROUP



* TRANSFERRIN SATN. <16%, ERYTHROCYTE PROTOPORPHY. >124.6 NMOL/L RBC, MCV <78 FL
SOURCE: AM J CLIN NUTR 1989; 49: 553-558

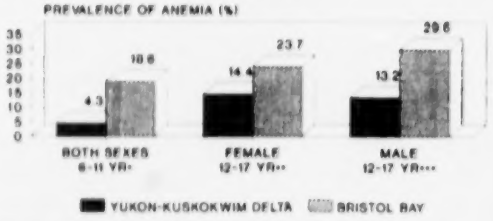
status was highest in non-Hispanic blacks. The results of this study suggested that the Hispanic groups studied were not at greater risk of impaired iron status than non-Hispanics.

In contrast to the relatively low prevalence of anemia reported for other ethnic groups, data from several sources show that the prevalence of anemia remains high among Alaskan Native children (17).

An anemia survey was conducted among 876 children from 15 villages in the Yukon-Kuskokwim Delta area, and among 318 children from 7 Bristol Bay villages. Figure 12 shows that the prevalence of anemia was much lower in the Yukon-Kuskokwim Delta area than in the Bristol Bay area. Overall, the combined prevalence of anemia was 23% for

Figure 12

PREVALENCE OF ANEMIA AMONG ALASKAN NATIVE CHILDREN



* HB <11.5 G/DL ** HB <12.0 G/DL
*** HB <12.5 G/DL
SOURCE: MMWR 1988;37:200-202

the Bristol Bay area, and 10% for the Yukon-Kuskokwim Delta. Serum ferritin levels were determined in a random sample of 83 children from the Yukon-Kuskokwin Delta; 65% had serum ferritin levels below 10 ng/ml. The cause of the continuing high prevalence of anemia and iron deficiency among Alaskan Native children, especially the older children, is unclear. There is a need for further research in this area.

Conclusions

It should be clear from this review that much is known about iron metabolism in infants and children. It should be equally clear that in the United States iron deficiency is preventable at a relatively modest cost, and that we have sufficient knowledge and the technology to do so. Fortunately, iron deficiency appears to be on the decline among most ethnic groups of infants and children that have been studied, one possible exception being Alaskan Natives. The decline in the prevalence of iron deficiency can be attributed to nutrition education efforts by nutritionists, dietitians and other health personnel, improved iron intakes, more effective iron fortification of foods, and the positive impact of public health nutrition programs such as the WIC Program. With a continuing decline in iron deficiency, we can all anticipate the eradication of this nutritional problem within our lifetime.

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IRON DEFICIENCY COEXISTING WITH SICKLE CELL ANEMIA

Clayton L. Natta
Department of Pathology and Medicine
Columbia University College of Physicians
Harlem Hospital, New York 10037

Introduction

Iron deficiency anemia (IDA) is the commonest anemia seen in clinical practice (1). Sickle Cell Anemia (SCA) occurs with an incidence in blacks of 1 in 600 live births in North America (2). The incidence of the gene is 40% in parts of Uganda(3) Therefore, the coexistence of these two conditions must occur fairly frequently. However, the cause of iron deficiency in SCA is usually not obvious, and the condition is difficult to diagnose, possibly because the iron deficiency is overshadowed by a chronic hemolytic process. SCA is a chronic hemolytic anemia due to the inheritance of an abnormal hemoglobin, Hgb S, in which valine replaces glutamic acid in the sixth position from the N terminal of the beta chain(4). Because of this change, HgbS becomes insoluble and polymerizes under conditions of hypoxia, acidosis, dehydration. The life span of the sickle erythrocyte is 17 days compared to a normal erythrocyte life span of about 120 days. In the cell, 90 percent of the hemoglobin is HgbS. The balance consists of HgbF and normal amounts of HgbA2.

The morphology is normochromic, macrocytic with moderate anisopoikilocytosis, and irreversible sickle cells (ISC) on smear. The latter comprise 4-44 percent of oxygenated erythrocytes in SCA(5,6). These cells play an important role in causing iron deficiency in SCA. Since sickling occurs when the blood is deoxygenated, most sickle cells are found in the venous circulation. Cells with more HgbS and less HgbF tend to sickle more easily and thus form ISCs.

Pathogenesis

In order to understand the extent of the problem of IDA in SCA, one should use as the point of reference, normal iron metabolism. In adult males and postmenopausal females, iron deficiency is due to blood loss. In adolescent patients, the requirement of iron for growth is an important factor in contributing to iron deficiency. Normally 10 percent of the iron in the western type diet is absorbed(7). In one study(8) three SCA children absorbed 57 percent of ingested iron compared to 13 percent in normal children. Because of exchange transfusions and other transfusion regimens, it is generally believed that iron stores are increased in SCA patients. One unit of blood delivers 250 milligrams of iron. Over a period of time one would expect the iron stores to be greater than the normal 1.2 - 1.5 grams. However, excluding obvious blood losses such as hematemesis and menorrhagia, the losses via the urine as hemosiderinuria play a significant role in producing a negative iron balance in some patients with SCA.

The ISCs are unable to revert to the biconcave shape on oxygenation. They have been postulated to have a membrane defect causing them to leak potassium and hemoglobin in the plasma and to accumulate calcium(9). Raised plasma Hgb levels and urine hemosiderin have been described in SCA patients (10). The free hemoglobin in the plasma combines with haptoglobin, an alpha-2-glycoprotein. The hemoglobin-haptoglobin complex determines the renal

threshold for hemoglobin(11). After the haptoglobin is saturated, free hemoglobin exists in the plasma. A small percent of the unbound Hgb is converted to methemoglobin which dissociates into ferriheme and globin. Ferriheme is bound by hemopexin(12), a betaglycoprotein. A fraction of the unbound hemoglobin in the form of alpha-beta dimers passes the renal glomerular membrane and is reabsorbed into the proximal renal tubules until the absorptive capacity is exceeded. Desquamation of tubular cells in the urine results in the appearance of urine hemosiderin(13) and loss of iron from the body. This loss may be significant in SCA. Washington and Boggs(14) reported that 27 of 35 SCA patients excreted abnormally high amounts of urine iron, five fold that of normal subjects. Urinary loss of iron is the major reason for iron deficiency in SCA, excluding obvious blood loss.

Diagnosis

The physical findings in IDA are superimposed on those of SCA, and are not helpful in diagnosis. For example glossitis is present in both conditions. The diagnosis is made from several studies which are usually necessary:
A) Serum ferritin, Serum iron, and percent saturation.
B) Bone marrow stain for iron.
A negative Prussian blue stain of bone marrow in SCA is not the sine qua non of iron deficiency. A case has been described in which the bone marrow iron stain(15) is negative, but there is iron in the liver and kidney. This phenomenon has also been described in autoimmune hemolytic conditions as inflammation affecting the ferritin levels, the presence of a thalassemia gene, or abnormalities of the liver

Case Reports

There have been many reports of IDA in SCA (17 - 23). In several, the diagnosis is made by one or more of the methods outlined above. In some reports, the diagnosis may not follow the same rigid guidelines from institution to institution. In a report(23) of a 46 year old patient with iron deficiency, the diagnosis of SCA is in question, because the reported Hgb A2 of 4.6 percent supported instead the diagnosis of sickle beta-thalassemia.

Two autopsy studies are worthy of mention. In a 26 year old male homozygous S patient who was on an exchange transfusion regimen during childhood, iron deficiency was diagnosed prior to death. The peripheral smear showed hypochromia and microcytosis. There was a low serum iron, a 10 percent saturation of transferrin, normal Hgb A2 and Hgb F levels, and a low serum ferritin. At autopsy, there was no gastrointestinal lesion, nor was there any iron in the liver, spleen, bone marrow or kidney. Rao et al reported the case of a 47 year old SCA patient in whom iron deficiency was diagnosed before death, by low serum ferritin and low serum iron. At autopsy there was no stainable iron in the bone marrow, liver, or heart. Iron, however, was found in the kidneys and atrophic spleen, but apparently was not available for reutilization.

Potential beneficial effect

SCA patients with coexisting iron deficiency have been reported to be mild clinically. In one report (24), 3 SCA patients overtly iron deficient, had no painful crises until they were supplemented. In one of the patients (25), the 51CrT50 decreased from 15.9 days while iron deficient to 5.2 days after supplementation. Such reports support the observation of Greenberg et al(26) that a mean corpuscular sickle hemoglobin concentration of 15-18 gm per deciliter, correlated with a mild hemolytic state, whereas significant anemia resulted from a mean corpuscular sickle hemoglobin concentration of over 20 gm per decimeter.

Many researchers in the field(27), regard the intracellular gelation of deoxyhemoglobin S as the primary pathologic change in SCA. The first stage is the delay time which is very sensitive to the deoxy Hb S concentration and temperature. The second stage is a sigmoid progress curve. A decrease in the concentra-

tion of Hgb S by 1 gm/dl increased the delay time by a factor of 4. It has been calculated that a decrease in the mean corpuscular sickle hemoglobin concentration of 4 gm per deciliter may increase the delay time by a factor of 1000. This has been postulated to produce a therapeutic effect by decreasing clinical severity.

This concept was modified and tested by several researchers with negative results. They all tried to artificially decrease the MCHC, Rosa et al(29) reported that in an attempt to achieve a therapeutic goal in three SCA patients, they induced a sustained dilutional hyponatremia by the use of an analog of arginine vasopressin, a low salt diet, and high fluid intake. The MCHC fell from 34 to 30 percent. These researchers felt that they were able to shorten the duration of crises. Leary and Abramson (3) in a letter, reported that in their study using this technic, there was little benefit for the patients, and greater concern that the method was potentially toxic. The experience of Charache et al(31) following a similar procedure was that this method of treatment did not decrease the SCA patients' stay in hospital and was fairly costly since it required constant laboratory backup. Most SCA researchers agree with Lincoln et al(32). It is conceivable that other methods of manipulation to lower the MCHC are possible, and should be sought.

Abstract

In Sickle Cell Anemia (SCA), reversible and irreversible sickled cells leak hemoglobin in the plasma, with the eventual formation of hemosiderin in the urine. The loss of iron has been reported to be as high as five or six fold that of normal individuals. Several reports of iron deficiency in SCA exist in the literature, reflecting it to be a fairly common event. Few anecdotal reports describe SCA patients, when iron deficient, to be much milder clinically than when iron supplemented. This is consistent with the calculation that a fall in the mean corpuscular sickle hemoglobin concentration of 4 gms per dl would increase the delay time by a factor of 1000. Thus far, the manipulation of the MCHC by dilutional hyponatremia has failed to decrease the duration or severity of SCA crises.

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IRON DEFICIENCY, THYROID FUNCTION, AND THERMOREGULATION

John Beard
The Pennsylvania State University
Nutrition Department
University Park, PA 16802

Iron deficiency is a major nutritional concern in developed and developing countries around the world with approximately 15% of the world's population having iron deficiency anemia (1). The Second National Health and Nutrition Examination Survey (NHANES II) indicated that, depending on the model of assessment used, 1-6% of the U.S. population has impaired iron status, including 3.5-12.1% of males 11-14 years of age and 2.5-14.2% of females 15-44 years of age (2). The manifestations of iron deficiency apart from clinical sequelae associated with severe anemia are alterations in immune function, cognitive performance and behavior, catecholamine metabolism, glucose metabolism, thermoregulatory capacity, and exercise and work performance (3-6).

Specific studies in rats and humans demonstrate failure in metabolism in iron deficiency that appears to be independent of tissue oxidative capacity. Nutritional iron deficiency anemia limits body temperature maintenance during acute cold (4°C) stress in rats (6-10). Iron deficient anemic people have significantly greater losses of core body temperature when cold stressed than controls (11) even when body fatness is accounted for (12 - 14).

It is also now becoming clear that the thyroid hormones and the sympathetic nervous system play important roles in both dietary and cold-induced thermogenesis (15). Numerous investigations (for review see References 16, 17) now show that facultative heat production is a mechanism to not only increase body heat during cold stress but also to buffer variations in dietary energy supply. Variations in the ability of individuals to vary this component of total energy expenditure can have a large effect on overall energy balance in certain situations. The importance of these observations on thermogenesis and iron deficiency gains new perspectives if one recalls that Garn (18) observed that iron deficient anemic adolescents were significantly leaner than their normal counterparts after covariation in age, sex, genetic ancestry, and socioeconomic status were controlled. This tentative link raises the possibility that iron deficiency anemia is related to altered caloric requirements due to neuroendocrine and metabolic dysfunction. Pertinent literature will be reviewed in two sections, namely thermogenesis and then iron deficiency.

Thermogenesis

Thermogenesis in homeotherms increases above an obligatory basal level whenever environmental temperatures drop below thermoneutrality. Norepinephrine (NE) and intracellular triiodothyronine (T_3) drive non-shivering thermogenesis in the rat primarily by sympathetic nervous system (SNS) activation of brown adipose tissue (16-18). In man, the former component is quantitatively less clear. Cooperative adjustments of both sympatho-adrenal and pituitary-hypothalamic-thyroid systems are required for appropriate thermogenic responses in brown adipose tissue and elsewhere (16,19). Hypothyroid animals have a lower BMR and require a higher SNS activity to maintain normal metabolic rate at any temperature below thermoneutrality. Maximal heat production is reduced and the thermoneutral temperature is elevated (16). Though earlier studies demonstrated a permissive role of T_3 in regulation of brown adipose tissue responsiveness to sympathetic nervous system

stimulation, more recent kinetic studies show a more active role of intracellular T_3 (20). These involve effects on up and down regulation of the alpha 1 and beta 1-adrenergic receptor sites, changes in synthesis of mitochondrial thermogenin, "uncoupling protein," and changes in the sources of plasma T_3 (21,24).

Attention in recent years has focused on the regulation of the conversion of thyroxine to triiodothyronine ($T_4 \rightarrow T_3$) in peripheral tissues (reviewed in 22). Two enzymes appear responsible for this deiodination. They are distinguished using two primary criteria, (a) the selectivity of the reaction catalyzed, and (b) inhibition by propyl-thiouracil (PTU). Type I 5'-deiodinase (5'-D-I) is located primarily in the kidney, liver, and skeletal muscle (with relative activities of 40x, 20x, and 1x, respectively) with activity levels similar to those found in the muscle also in spleen, lung and intestines. The type I deiodinase acts on both the inner ring (position 5) and the outer ring (the 3' and 5' positions are stereochemically identical) of the molecule while the Type II deiodinase (5'-D-II) is specifically an outer ring acting enzyme. 5'-D-II is probably plasma membrane associated and located primarily in the cerebral cortex, anterior pituitary, and brown adipose tissue. The Km for T_4 and rT_3 is lower in 5'-D-II than in 5'-D-I and is not subject to inhibition by PTU in vivo or in vitro (23).

5'-D-I activity decreases in hypothyroid states and increases in hyperthyroid states (18 - 20). Usually 80% of the extra thyroidal T_3 production is via 5'D-I production (19,20). In hypothyroidism, the 5'-D-II becomes the significant provider of T_3 to the plasma pool. T_4 is a potent and immediate inhibitor of the Type II BAT-deiodinase. T_3 produced within the BAT by this enzyme is a key regulator of "thermogenin" or "uncoupling protein" concentration in BAT (20) and maintains a high 80% nuclear T_3 receptor occupancy for maximal thermogenesis.

There is a body of literature that demonstrates that SNS activity is increased during cold exposure as demonstrated by increased efferent sympathetic nerve traffic and increased NE turnover rates in brown adipose tissue, and other organs (17,24,25). The numerous mitochondria in brown adipose tissue become uncoupled and heat production proceeds at a maximal rate (16). Associated with this increased SNS release of NE is increased alpha 1-adrenergic activation of 5'-D-II and increased "uncoupling protein" synthesis (19). Thus, it seems both the SNS and thyroid systems are critical for proper regulation of heat production (26).

Animal studies

Animal studies of thermoregulation in ID in the rat can be divided into investigations in the (1) CNS, (2) peripheral SNS activity, and (3) thyroid function. Within the CNS, certain enzyme systems pertinent to thermoregulation have been examined. Brain vesicular uptake of 5-hydroxytryptamine is impaired (27,28). Tyrosine hydroxylase, an iron dependent enzyme, does not appear to be decreased in ID. Others (29) have noted a defect in dopamine (D2) receptors in parts of the CNS but a clear functional consequence of iron deficiency in the CNS relative to thermoregulation is lacking.

In addition to the CNS studies, peripheral catecholamine metabolism also appears to be dramatically altered in iron deficiency. Elevated levels of NE in urine of iron deficient children (30) and rats (31) without cold exposure suggests a defect in NE catabolism and/or in synthesis.

Iron deficient anemic animals become hypothermic at 4°C and die if their hematocrit is <25% (7,8,32) (Figure 1). We (4) were able to amend some early observations (6) and to show that exchange transfusion corrects the abnormality in thermoregulation. What is apparent in all studies is that basal T_3 and T_4 levels are depressed in ID anemia. Acute exchange transfusion returned the ability of ID animals to generate an appropriate T_3 response and to have a much less significant decrease in core temperature. It is important

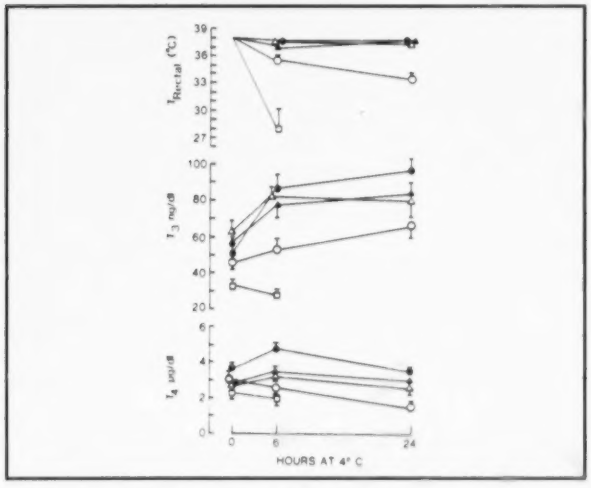

Figure 1. Changes in rectal temperature, serum triiodothyronine (T_3), and serum thyroxine (T_4) after cold exposure of iron-deficient ad control rats. Controls rats were exposed to cold before (•-•, n-14) and after (o-o, n=11) transfusion to hematocrits near 30. Some iron deficient rats on initial exposure to cold had a fall in temperature to less than 31°C and were removed at 6 g (▼-▼, n=8); others maintained temperatures above 31°C and remained in cold for 24 h (▼-▼, n=9). Iron-deficient rats transfused to hematocrits near 30 (▽-▽, n=8) maintained normal body temperature for 24 h of cold exposure. From Beard et al., Am. J. Physiol. 247:R114-R119, 1984.

to note the TSH response to cold also improved dramatically with exchange transfusion (Figure 2).

We recently extended the early observations of thyroid hypofunction in IDA with some modest experiments in three areas: (1) TRH stimulated release of TSH, (2) plasma T_3 disposal rate calculations, and (3) hepatic thyroxine-5'-deiodinase (5'-D-I) and BAT deiodinase (5'-D-II) enzyme activities. Briefly, iron deficient anemic rats have a significantly blunted TSH response to TRH injected at three physiologic doses (Figure 3). IDA rats also have a significantly slower plasma T_3 disposal rate (Figure 4) at room temperature. Calculated disposal of T_3 in CN's average 88 ng/d compared to only 42 ng/day in IDA's.

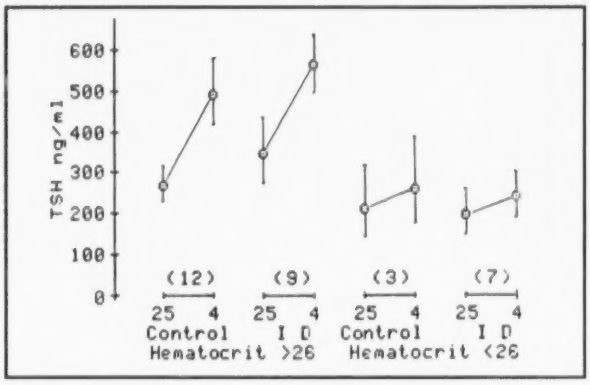

Figure 2. Thyroid-stimulating hormone (TSH) response to cold exposure. Paired results at 25 (o) and at 4°C (o) are depicted as geometric means ± SE computed after logarithmic transformation. When hematocrit (HCT) exceeded 25, a significant increase occurred both in control (P<0.001) and iron-deficient (P<0.001) and iron-deficient (P<0.025) rats. In aals with lower hematocrits, TSH did not rise significantly. From Beard et al., Am. J. Physiol. 247:R114-R119, 1984.

Table 1

	Liver - Type I-deiodinase (pg T_3/mg protein/min)[a]			IBAT - Type II-deiodinase (pg T_3/mg protein/min)		
	30°c	10°c-24 hrs	10°c-72 hrs	30°c	10°c-24 hrs.	10°c-72 hrs.
IDA	37 ± 10*	30 ± 5*	144 ± 18*	23 ± 8*	63 ± 12*	113 ± 11
CN	91 ± 15	127 ± 8	176 ± 10	11 ± 6	100 ± 18	97 ± 14
Number	8/grp.	10/grp.	8/grp.	8/grp.	10/grp.	8/grp.

[a]IDA rats had Hb=4.6 ± 1.2 gm/dl and weighed 220 ± 20 gms while CN's had Hb=14.5 ± 1 and BW=310 ± 29 gms; 5'-D-I was measured in clarified liver homogenates using published methods.
*Activity was significantly different (p<.05).

These data agree quite strongly with T_3 disposal rates previously observed in hypothyroid rats (19,20). Plasma T_3 production is approximately 70-80% from the 5'-D-I in liver and kidney in euthyroid rats and approximately 50% in humans. In hypothyroidism, hepatic and kidney T_3 production declines very rapidly while the 5'-D-II becomes the major donator to the plasma pool (21).

With this in mind, we performed additional studies of Type I and II-5'-D activities in IDA rats exposed to 30°C or 10°C for various periods of time (Table 1).

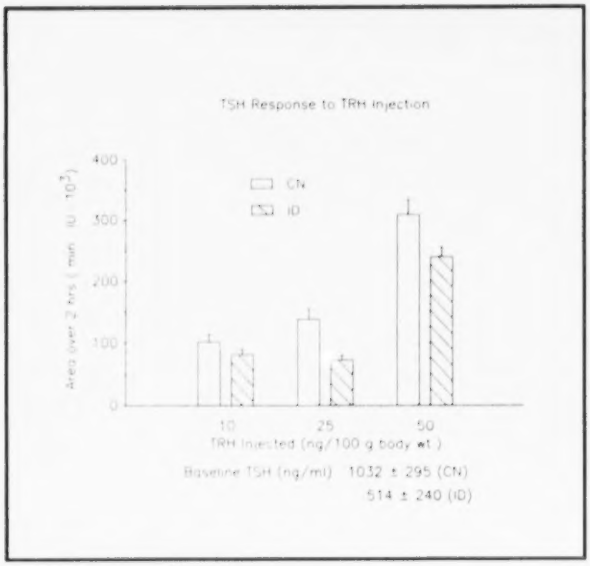

Figure 3. Plasma TSH response as area under a 2 h curve ($\bar{x} \pm SE$). ID and CN rates were injected with 10, 25, or 50 ng/100 g BW TRH in sterile saline. From Beard et al., J. Nutr. 119:772-779, 1989.

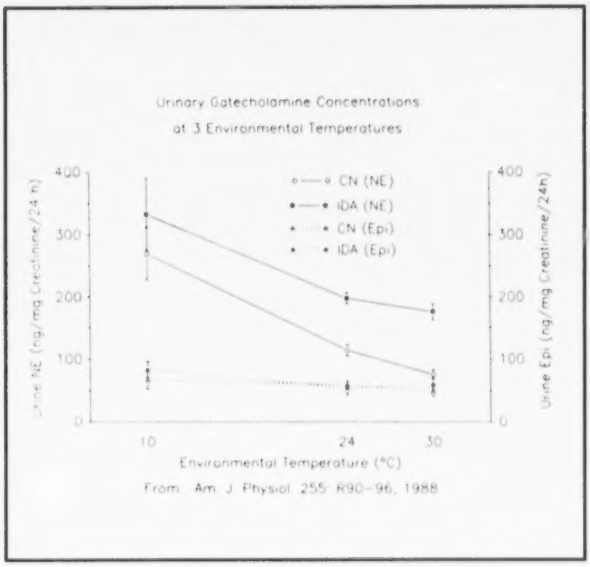

Figure 5. 24 h urine norepinephrine (NE), epinephrine (E) in iron deficient and control rats after 7 days at one of three environmental temperatures. From Beard et al., Am. J. Physiol. 255-R90-R96, 1988.

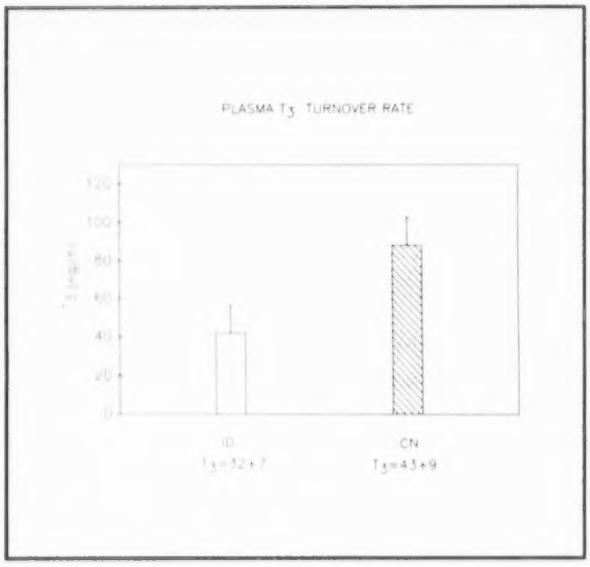

Figure 4. Plasma T_3 turnover rate ($\bar{x} \pm SD$). Plasma concentrations are shown below. From Beard et al., J. Nutr. 119:772-779, 1989.

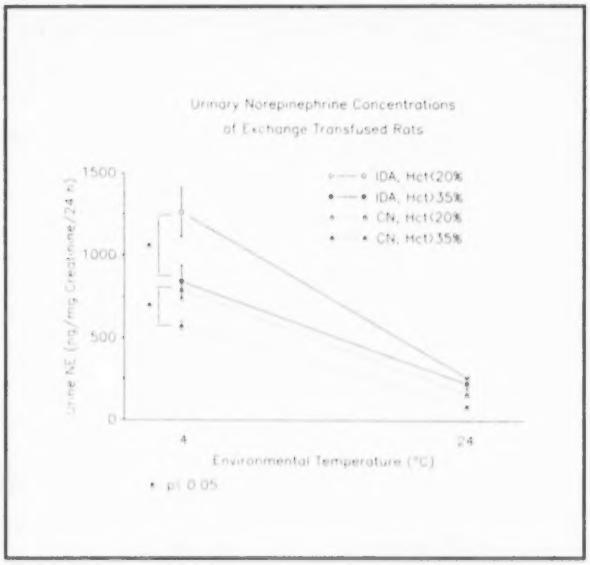

Figure 6. 24 h urine catecholamines in ID anemic rats with hematocrits <20% and exchange transfused to Hct >35% at 2 temperatures. Controls were also exchange transfused to Hct <20. n = 8-12 per group.

IDA animals were significantly below CN's in hepatic 5'-D-I enzyme activity with the greatest difference occurring with acute cold exposure. We had already shown that the T_3 rise was very sluggish in IDA's in this paradigm. After 3 days, however, IDA's were able to increase 5'-D-I in liver to activities much closer to controls. If a slightly smaller liver:BW ratio in IDA's is considered, total hepatic production of T_3 is still 35% less in IDA's than controls. The second extrathyroidal donor to the plasma T_3 pool, IBAT 5'-D-II, also has a delayed response. The greater reliance on the Type II-5'-D at the near thermoneutral 30°C temperature agrees with the concept that IDA's are hypothyroid animals and produce most of their plasma T_3 from this pathway. The actual proportion of circulating T_3 derived from 5'-D-II, however, remains unknown in ID.

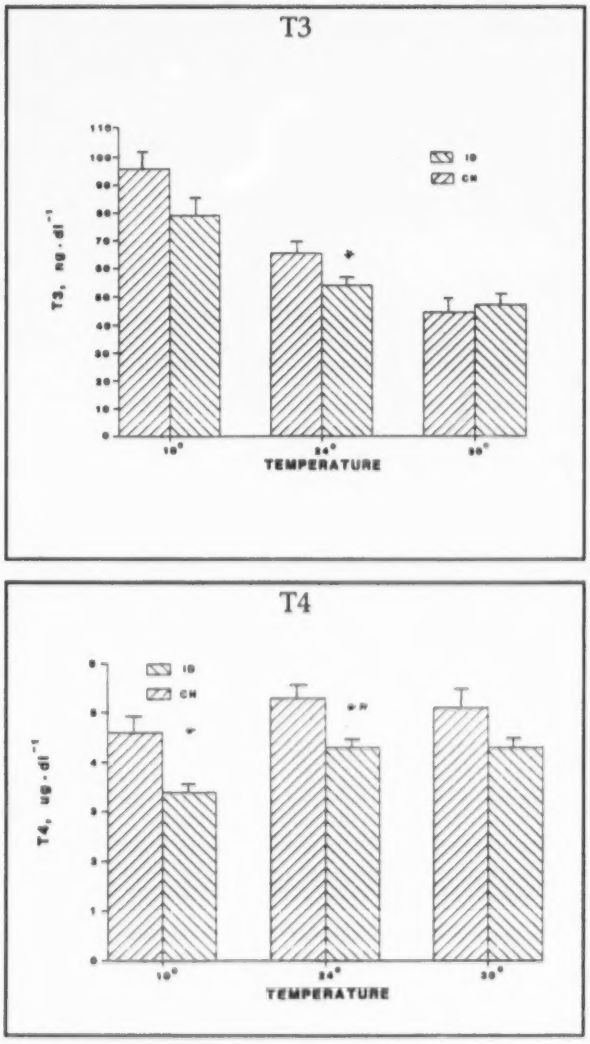

Figure 7b. Plasma T3 and T4 concentrations in ID and CN rats at three temperatures. From Beard et al., Am. J. Physiol. 255:R90-R96, 1988.

The hypothesis of increased secretion rates of NE to compensate for decreased peripheral conversion of $T_4 \rightarrow T_3$ was proposed by Dillman et al. (9) These conclusions were based primarily on bolus T_4 versus T_3 replacement in thyroidectomized animals. High dose T_3-replaced animals could withstand the cold, while T_4-replaced animals could not. We now know that the large dose of T_4 administered was a potent inhibitor of 5'-D-II, thus decreasing the likelihood that non-pharmacologic based conclusions could be drawn. ID anemic animals at 4°C had hypothermia, lowered T_3 levels, and decreased metabolic rate despite hypernorepinephrinemia.

The initial experiments of Dillman (9) also showed slowed rates of clearance of ^{3}H-NE from the plasma pool in IDA animals. Since the rate of disappearance of the labelled NE tells us little about tissue metabolism, we examined NE turnover in iron deficiency as a function of environmental temperature (10). We employed an animal model of 7 days exposure to 30°, 24°, or 10°C to examine the effect of chronic environmental temperature on general catecholamine metabolism (Figure 5). Near the thermoneutral temperature of 30°C, IDA rats have significantly more NE excreted/day per gram of creatinine compared to controls, while at 10°C, this difference is negligible. HVA, at a concentration 10 times greater than the free catecholamines, is consistently elevated in ID rats (10). Acute exposure does lead to increased urine NE that is not corrected with exchange transfusion (Figure 6). This difference in urinary NE response in the acute cold exposure experi-

ments (when NE is much greater in ID) and longer term exposure (where NE is similar in ID and control) may reflect changes in secretory rates, catabolic rates, and SNS responsivity. Through accurate quantification and metabolite isotope tracing we can begin to address this issue.

Brown adipose tissue NE turnover was significantly altered by IDA at all temperatures (Figure 7). Most importantly, there was a pronounced failure of iron deficient rats to increase turnover at 10°C compared to the rate at 24°C. Since increased nerve traffic and NE turnover are the normal responses, it is quite likely this represents a clear defect in stimulation of heat production. Dillman (32) also reported a significant increase in resting oxygen consumption of ID animals at 25°C and 30°C, but a decreased consumption at 4°C. As there were few animals in those studies and some problems may have existed relative to the measurement technique, a careful re-examination has been conducted. We recently carefully examined resting VO_2 in age matched IDA and controls at 5 different temperatures (33). Measurements were made with an open circuit indirect calorimeter. There were significant elevations in VO_2 per kg body weight in ID compared to controls at all temperatures less than 30°C. Importantly, pharmacologic injection of NE to achieve VO_2max showed higher, not lower, maximal NE heat production. Thus, anemic animals appear hypermetabolic and hypothermic at the same time. As we will see, this is similar to the initial human studies.

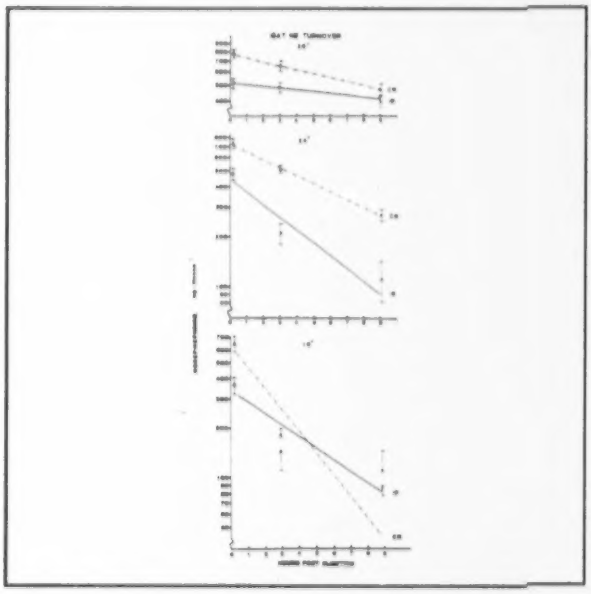

Figure 7a. Interscapular brown adipose tissue norepinephrine (IBAT-NE) turnover in ID and CN rats at three temperatures. From Beard et al., Am. J. Physiol. 255:R90-R96, 1988.

Human studies

The published literature in this area is limited in human subjects. One early preliminary study (11) in Venezuela observed that iron deficient anemic subjects exposed to 28°C water for 1 hr. became colder than controls, had higher metabolic rates at rest and through the cold exposure, and had significantly higher plasma catecholamines (Figure 8). The interpretation of this study was limited, however, due to the thinness of the IDA's relative to iron sufficient controls. This limited the interpretation since the poor thermoregulation could be attributed to the low body fatness of the IDA. In addition, it raises the interesting possibility that leanness was

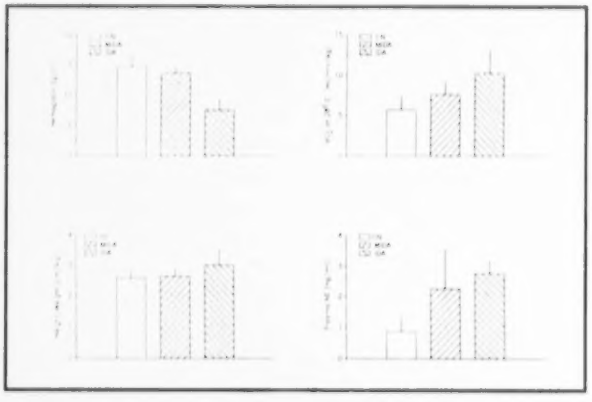

Figure 8. VO_2, plasma norepinephrine, hemoglobin of subjects exposed to 28° for 1 hr. Adapted from Martinez-Torrez et al., Am. J. Physiol. 246: R380-R383. 1984.

causally related to iron deficiency. This latter point has now been clearly demonstrated in laboratory animals in which IDA rats were significantly less calorically efficient than controls (34) (Table 2).

Table 2

	Hb gms/dl	BW change gms	Efficiency % retained
Iron deficient anemic (n=64)	6.5 ± 6	30 ± 8*	25.6 ± 4.2*
Control (n=32)	11.5 ± 1.2	43 ± 10	31.0 ± 5.0

*Age matched Sprague Dawley rats fed low iron <3 ppm Fe from day 21 to day 50 or control diet (55 ppm Fe); P <.001 difference, BW change and feed efficiency over a 7 day period.

Two additional human studies have been completed. One study was conducted at the USDA Human Nutrition Research Center in Grand Forks, ND by Hank Lukaski and involved six female subjects who consumed iron-depletion (80 days) and iron-repletion (100 days) diets (14). These subjects underwent an 18°C cold air tolerance test following each diet phase. Iron status measurements and body temperature were significantly lower while plasma NE concentrations were significantly greater during cold exposure in the iron depletion than in the iron repletion phase. There was also the tendency for the IDA women to have lower T_3 and T_4 concentrations than controls. Thus, these findings support an impaired thermogenic capacity in iron-deficient subjects.

Another study, conducted by our laboratory at Pennsylvania State University, used a cold (28°C) water (12,13,35) stress. In brief; IDA women with the same body fatness as controls were significantly colder, were hypometabolic, and had significantly lower plasma T_3 and T_4 concentrations than controls.

Prior to iron supplementation, the iron-deficient anemic women had an impaired thermoregulatory capacity compared to the con-

Figure 9. VO_2 at 100 minutes of 28°C water exposure in control (n= 8) and tissue iron deficient (TD, n=12) subjects prior to (pre) and after (post) 12 weeks of iron supplementation.

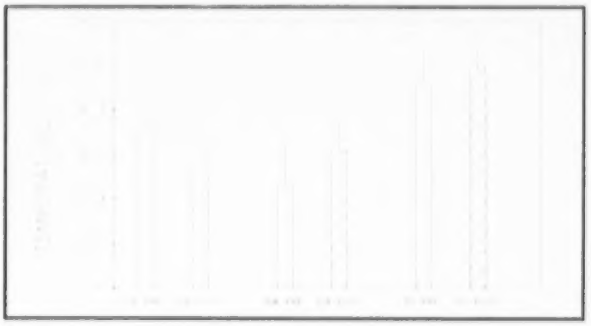

Figure 10. Rectal temperature response at 100 minutes of 28°C water subjects as in Figure 9.

Figure 11. Plasma T3 concentration at 100 minutes of 28°C water bath. Subjects as in Figure 9.

trol women as indicated by a significantly greater decline in mean body core temperature (Figure 9) after 100 minutes of cold exposure and a lesser increase in VO_2 as shown in Figure 10. These differences were significant within 40 minutes of exposure to 28°C. In addition, the mean plasma T_3 and T_4 concentrations of the iron-deficient anemic women were significantly lower than those of the control women at baseline and throughout the cold exposure (Figure 11). After iron supplementation, the anemia was corrected and thermogenic performance was improved as indicated by a significantly higher body temperature after 100 minutes. Thus, the two recent independent studies are in very good agreement and demonstrate a clear alteration in thermoregulation and thermogenesis in IDA with a possible basis in thyroid dysfunction and catecholamine metabolism.

In conclusion, these experiments in both humans and rats demonstrate a clear metabolic defect in thermoregulation in iron deficiency. The human and rat data both suggest thyroid hormone dysfunction may underlie the thermoregulatory failure and suggest that anemia per se is a significant factor. In contrast, alterations in SNS activity are far less consistent and require more detailed organ based experimentation.

I would like to acknowledge the financial support of the National Institutes of Health, the extramural program in Human Nutrition of the USDA, and the Pennsylvania Heart Association. Importantly, I also acknowledge the dedicated work of Brian Tobin, Myfawny Borel, Scott Smith, Steve Smith, Dale Brigham, Karen Glyde and Nancy Hopkins who have made many of these observations possible.

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IMMUNITY AND INFECTION IN IRON DEFICIENCY

Gerald T. Keusch
Division of Geographic Medicine and Infectious Diseases
New England Medical Center, Tufts University School of Medicine
Boston, MA 02111

Nutritional status and its effects on susceptibility to infection, host defense mechanisms, and clinical immunology in humans continues to be a very controversial subject. By carefully picking the presenter at a conference or the author of a paper or chapter, it is possible to preselect polar views on almost any topic in the field. Nowhere is this more apparent than for iron deficiency, which is not only among the most prevalent nutrient deficiencies in the world, but one that is associated with population groups with high prevalence of infection, including the very young, the old, and those with protein-energy malnutrition. One resulting problem is that because a causal association will often be attributed to two high frequency events occurring in the same population, iron deficiency may be considered to be a cause of high rates of infection. However, proving this can be remarkably difficult.

It is important to recognize at the outset that resistance to infection is not an all or none phenomenon (1). The outcome of any encounter between host and infectious agent ultimately must be measured in quantitative terms, since a dose-response curve exists relating how many organisms are needed to infect a defined percent of an exposed population and how many of those infected will become ill. The results differ from organism to organism and among strains of the same organism, and often from population to population as well. These data in general show that while a small inoculum may be contained by the host, a larger dose of the same organism will cause infection and illness. Even immunization does not represent an absolute barrier, and in addition to primary and secondary vaccine failures, where immunity either fails to develop in the first place or wanes after some period of time, the effect of a vaccine is to shift the dose response curve to the right so that a larger inoculum is necessary to cause disease in the immunized host. The quantitative dimension applies also to response time of host defenses, and the balance between resistance and susceptibility may be shifted if an individual cannot mount an effective immune response soon enough after encounter with a pathogen. These are particularly challenging parameters to measure in humans.

Table 1

DETERMINANTS OF SUSCEPTIBILITY TO INFECTION

INNATE RESISTANCE

ACQUIRED RESISTANCE

INOCULUM SIZE

ROUTE OF INFECTION

AGE OF HOST

INTEGRITY OF BARRIERS

UNDERLYING DISEASE

NUTRITIONAL STATUS

Table 2

ASSESSING THE NUTRITION-INFECTION INTERACTION

NUTRITION	IMMUNE FUNCTION	SUSCEPTIBILITY
clinical	in vitro	field study
biochemical	in vivo	
epidemiologic		

A number of factors thus condition host resistance (Table 1). Another determinant of susceptibility is nutritional status (2,3). While it is clear that nutrition affects host susceptibility and response to infection, usually in a negative manner (4-6), and that this is largely determined by nutritional effects on the immune system and host defenses (1-3), it remains difficult to quantitate the risk of infection due to malnutrition because it is so hard to relate quantitative measures of immune function in individuals to qualitative assessments of susceptibility in populations (Table 2) (7). The difficulty is not so much in assessment of nutrition, for which clinical, biochemical and epidemiological methods are now available, or even in application of clinical immunology tests used to assess immune function and status of host defense, for this can be done by *in vitro* and to some extent *in vivo* tests, but rather to assess susceptibility, which requires a large population and prospective standardized measures of morbidity, applied with a sufficient frequency to determine all new episodes and accurately assess their duration and severity, over a long enough time to permit a "representative" exposure to pathogens (which varies over short periods of time in all populations because of a variety of epidemiological factors independent of the presence of an "at risk" population), and to control for confounding factors other than nutrition which affect susceptibility (1).

Iron deficiency: the paradox

When the epidemiological data and the results of certain clinical studies have been examined, divergent conclusions concerning the interaction of iron status and infection have been reached (8). Two major camps have developed. In one, the view is that iron deficiency impairs immune responses and by means of this increases susceptibility to infection. The defect may therefore be repaired by iron replacement. The second camp concludes that iron deficiency is a state of natural defense to infection because microorganisms will have diminished ability to obtain the iron they require for metabolism and growth. The logical extension of this view is that iron replacement will generally help the infectious agent to the detriment of the host. Both views are based on data, and the differences are therefore in interpretation. The importance of reaching a balanced view is that questions of great public health concern hang in the balance (8,9). When should iron deficiency be treated? How should this be done? What preventive measures are appropriate? Do the choices differ according to age, presence of associated deficiencies such as protein-energy malnutrition, or clinical parameters? In addition, there are concerns about excessive iron and its effects on host susceptibility (8-11). Iron therapy may lead to different effects in malnourished hosts who are unable to control levels of free iron because of a lack of sufficient iron binding transport proteins.

The biochemical data base

All living organisms require iron for survival and growth (12). Humans, like microorganisms, use iron binding proteins to transport iron around from one site to another for efficient utilization. These proteins, transferrin and lactoferrin in the human, and phenoxylate or hydroxymate siderophores in microorganisms, have an extraordinarily

high affinity for iron and serve to trap and make available the needed iron. Siderophores are usually synthesized under conditions of limited iron availability in vitro, and respond to the environmental conditions. In the mammalian host, an early response to the stimulus of infection (or non-infectious inflammation) is the rapid transfer of iron from the circulation to an intracellular compartment, mediated by the cytokine interleukin-1 (IL-1) (13-15). This has been interpreted as a natural and non-specific antimicrobial defense tactic of the host to deprive the pathogen of the iron it needs, a mechanism sometimes described as "nutritional immunity" (16). However, recent data suggest that microorganisms grow in vivo under conditions of limited iron availability (17-21) and it is unclear how much significance should be attached to the "nutritional immunity" of iron sequestration by the mechanism of iron withholding (22).

It is known that certain virulence attributes of microorganisms are iron regulated. For example, toxin production by *C. diphtheriae* and *S. dysenteriae* type 1 is closely regulated by iron (23,24). In both organisms, production of toxin *in vitro* is maximum under conditions of relative iron deprivation and is shut off when iron is added to the media. This effect is mediated through the *fur* gene (25,26), which regulates siderophore production as well (27). When sufficient iron is present in the medium, the *fur* gene product binds iron and acts as a repressor of the promoter for siderophores and the toxins. If these systems can act analogously *in vivo*, then iron deficiency will prevent this negative feedback response. The result will be that at the same time as the siderophore is synthesized to compete for iron, toxin will be made as well with its subsequent ill effects on the host. One clear message in this is that *in vivo* systems can be far more complex than initially meets the eye.

Iron deficiency is reported to have a number of effects on immune system functions (Table 3). However, it must be remembered that iron deficiency in humans can be due not only to inadequate iron intake, but also to poor iron absorption or blood loss, each of which may be associated with factors other than iron that alter immune function. Thus, malabsorption of iron often goes together with malabsorption of other critical nutrients affecting the immune system, while blood loss due to parasitic infections or tumors can directly or indirectly alter immune function. Iron serves a role in many diverse enzymes and metabolic co-factors (28), such as cytochrome C, cytochrome C reductase, cytochrome oxidase, aconitase, succinic dehydrogenase, various peroxidases, catalase, xanthine oxidase, and ribonucleotide reductase. Not only is there an adverse effect of iron deficiency on oxygen transport and mitochondrial oxidative metabolism (28), but also inhibition of protein and DNA synthesis as well (29,30). Since a number of host defense functions depend on oxidative metabolism or cell proliferation, it stands to reason that iron deficiency could impair these mechanisms.

Table 3

IMMUNE FUNCTION IN HUMAN IRON DEFICIENCY

Consistent Changes

- o Mature T Lymphocytes in Circulation (E-rosetting cells)
- o Skin Test Reactivity (Candida, PPD, SK-SD, Mumps, Tricophyton, Diphtheria, Tetanus Toxoid, DNCB)
- o Proliferative Response to PHA
- o Phagocyte Myeloperoxidase
- o NBT dye reduction

No Consistent Change

- o Complement
- o Immunoglobulin level, including sIgA
- o Neutrophil bactericidal activity

Clinical immunological studies on iron deficient subjects have provided variable results, however. The most consistent abnormalities have been in cell mediated immune responses. In 1972, Joynson et al (31) found impaired skin test responses to *Candida* antigen and PPD, and subsequently noted the reversibility of the abnormalities with iron treatment (32). Chandra and Saraya (33) also described reduced numbers of E-rosetting cells in patients with iron deficiency, and noted impaired skin test responses and depressed *in vitro* lymphocyte proliferation in response to the mitogenic plant lectin, PHA, although no statistical analysis to support the conclusions was presented. In the same year, MacDougall et al (34) and Bhaskaram and Reddy (35) reported similar findings for skin testing and *in vitro* responses to PHA. The latter paper included data following iron treatment, showing an improvement in skin test responses. However their controls were not matched for age or sex, and the presence of infection was not reported. In 1976, Srikantia et al (36) found significant reductions in E-rosetting lymphocytes in severely iron deficient anemic children compared to mildly anemic or non-anemic subjects. However, no data were reported to rule out other causes of anemia. A few years later, Wakabayashi and Takaku (37) observed similar defects in PHA responses and were able to correct the abnormalities by addition of iron to the *in vitro* culture. Further studies by Talwalkar et al (38), Apostolov et al (39) and Maraes-de Souza et al (40) were consistent with these findings. Still, several other groups have reported negative findings. These include studies by Kulapongs et al (41), Coovadia et al (42), Gross et al (43), Suskind et al (44), and Grosch-Warner et al (45). While the reasons for the differences in results are not certain, some of the differences may be due to patient or control selection, the presence of recent or concomitant infection, or to variability in the laboratory methodology employed. The balance of the data indicates that cell mediated immune responses dependent on proliferation of T lymphocytes is likely to be impaired in relation to the degree of iron deficiency present, and suggests that the mechanism may involve impaired DNA and protein synthesis due to depressed activity of the iron dependent enzymes required for these processes. When many of the confounding factors are in fact controlled, and a state of "pure iron deficiency" is studied, defects remain which are reversible by iron administration, however their magnitude is not great (46).

Studies of other facets of the immune system, with the exception of the measurement of leukocyte myeloperoxidase, reveal no consistent defects and it is hard to escape the conclusion that iron deficiency per se does not affect antibody production, complement activity, or phagocytic cell function. There is however, a real deficit in the activity of another iron dependent enzyme, myeloperoxidase. Myeloperoxidase functions in a bactericidal system involving a halide and hydrogen peroxide produced during the oxidative burst associated with phagocytosis. It is one of several bactericidal systems possessed by the phagocyte, but because of this redundancy of microbicidal mechanisms in these cells, little effect is seen on susceptibility to infection even in patients with congenital myeloperoxidase deficiency, severely depressed enzyme levels, and diminished neutrophil bactericidal activity in vitro (47).

Evidence that iron deficiency promotes infection

One of the earliest observations of increased infection associated with iron deficiency states is that of Mackay (48), who treated nutritional iron deficiency in infants and noted a significant decrease of around 50% in the common respiratory and diarrheal diseases in both breast - and formula-fed babies. There are serious design deficiencies, however, in particular the non-random selection of study groups and the failure to match for a number of health related parameters. Subsequent studies have similarly been flawed with particular reference to randomization, failure to blind observers as to

iron status or therapy, lack of concurrent controls or use of historical control data, and too short a period of observation (49-54).

In some studies, iron deficiency has been detected in subjects who are identified because of infection. This has been interpreted to mean that iron deficiency predisposes to infection. However, it is well documented that infection is the cause of "iron deficiency" anemia due to cytokine mediated removal of iron from the active metabolic compartment. Thus, urinary tract infection has been reported to be more than twice as common in anemic pregnant women than non-anemic pregnant controls (55), and meningitis and pneumonia are each significantly associated with iron deficiency in infants hospitalized in Papua New Guinea (56). The problem is that it is not possible to exclude the possibility that infection is the cause of the iron deficiency anemia and not the other way around.

Does iron deficiency protect against infection?

As early as 1882, Trousseau (56) reported that iron administration could lead to clinical recrudescence of quiescent tuberculosis. Almost 100 years later, McFarlane et al (57) reported that administration of iron to subjects with severe kwashiorkor was frequently associated with a rapid demise. They suggested that in the presence of the depressed transferrin levels due to the PEM, administered iron was readily available to microorganisms and thus served to promote infection. Later, Masawe (58) found that iron deficient patients had many fewer diagnosed infections compared to those with anemia of other etiology (7% vs 65%). Malaria was found more commonly in the iron deficient subjects, but clinical attacks were noted to occur when iron administration was begun. Murray et al (59-61) made similar observations among Africans with dietary iron deficiency or following refeeding after famine. They reported an increase in smear positive malaria, increasing titers of antibody to *Brucella*, and more draining acid fast-positive scrofula lymph nodes following therapy with iron. Among the Somali nomads under study by these workers, 13 cases of malaria were documented during a 1 month period of oral iron compared to one case in the controls. An increase in smear positive malaria was also noted in a group of infants in Papua, New Guinea given parenteral iron at 2 months of age, along with a greater spleen rate as an index of increased incidence of clinical malaria (62). In contrast, there was no increase in malaria severity as judged by mortality rates, parasite density, or any increase in prevalence of cerebral malaria. However, a larger placebo controlled study in the same region of Papua, New Guinea designed to evaluate the effect of oral iron therapy for iron deficiency on malaria failed to find any effect on parasite prevalence, density of parasitemia, spleen rate or antibody detected by an ELISA method among older children (63). To control for the effects of iron, the effect of iron chelator, desferrioxamine, has been evaluated in *P. berghei* malaria infected rodents (64). Both *in vivo* and *in vitro*, free but not iron complexed desferrioxamine inhibited the growth of malaria in erythrocytes. In addition, there also was no evidence that desferrioxamine induced an iron deficient state *in vivo* (no reduction in serum iron, transferrin saturation, hemoglobin synthesis, or erythrocyte indices). No effect on the experimental malaria was found when iron levels were altered *in vivo*, by either inducing iron deficiency or by iron administration to produce an acute overload state. The most likely explanation for the *in vivo* efficacy of the chelator is therefore a direct action on the parasitized erythrocyte, effecting a decrease in iron availability for the parasite within red cells. This suggests that there is most likely a chelatable pool of iron within the erythrocyte.

Effects of iron administration and iron overload

A number of studies have reported that iron administration, usually in the form of parenteral iron-dextran, increases the rate of

severe infection in young infants (65-69). Together with clinical data suggesting an increase of certain infections in patients with diseases characterized by iron overload, the data have been extrapolated to mean that a state of iron deficiency will be protective. For example, when routine parenteral iron administration is used to prevent infantile iron deficiency anemia in New Zealand, an increase in the incidence of neonatal sepsis, in particular *E. coli* meningitis, is noted (65-67). When the practice is stopped, the rate of infection drops (68). Although these studies are flawed because of the lack of concomitant controls and only a short period of observation, the authors have vigorously defended their data and interpretation (69). More recent, better controlled studies in Papua New Guinea suggest that parenteral iron does have come adverse effect on the host (62,70). Specifically, the rate of severe lower respiratory infections and malaria increased significantly in 2 month old infants given prophylactic iron-dextran, and this group was also hospitalized more frequently and for a longer average stay. None the less, mortality rates were not different in the iron treated and control subjects, and the majority of the deaths were related to respiratory infections secondary to measles or pertussis. Interpretation is also complicated by the results of other studies of parenteral iron administration in young children, which have either observed no effect on infection prevalence rates or an actual decrease in infectious diseases in the treated subjects (71,72). On balance, it seems probable that parenteral iron in neonates is associated with increased incidence of severe infection, perhaps primarily in higher risk infants. Unfortunately, this will never be satisfactorily proven without additional placebo controlled studies, and it is probably impossible to conduct such a controlled study now.

Inferences about the adverse effects of excessive iron have also been made on the basis of observations made in patients with iron overload states, for example, hemachromatosis, a disease in which transferrin saturation is high and low molecular weight iron loosely complexed with albumin is present in the circulation and therefore readily available to invading microorganisms. While the lethal complications of this disease are largely due to organ dysfunction of the heart, pancreas (islets of Langerhans) or liver, however a significant mortality can be attributed to infections such as pneumonia (73). An unusual prevalence of *Y. enterocolitica* infections has recently been described in these patients (74-76), and an association, while not proven, is certainly suggested. *Yersinia* also emerges as a frequent pathogen in patients ingesting large amounts of iron as well as desferrioxamine (77,78). A possible basis for this may relate to the inability of *Yersiniae* to synthesize siderophores, even under conditions of iron deficiency. Thus, provision of excess iron could very well enhance growth and invasiveness of the organism. The occurrence of *Yersinia* infection in patients receiving desferrioxamine to complex the iron is explicable as well, since these organisms can use iron-desferrioxamine as a growth factor (79). In fact, *in vivo* experiments in mice demonstrate that the chelator is even more efficient than iron in stimulating the growth of the organism and enhancing virulence under iron-limiting conditions (79).

Sickle cell anemia is also associated with iron overload because of the large number of transfusions these patients receive. In fact, any hemolytic anemia is associated with increased serum iron from erythrocyte destruction, including thalassemia, malaria, and bartonellosis. A relationship between hemolytic states and systemic salmonellosis has been known for many years (80,81), but the critical features appear not to be the availability of iron, but rather the induction of mononuclear phagocyte cellular dysfunction due to monocyte ingestion of cellular debris (82). This form of "reticuloendothelial cell blockage" would account for the association of *Histoplasma capsulatum* infection of macrophages with heightened susceptibility to salmonellosis in the absence of hemolysis (83). These data indicate that it is dangerous

to extrapolate the presence of altered iron status in patients with increased susceptibility to infection, since the latter may or may not be causally related to iron status per se.

Summary

The data reviewed in this paper indicate that iron deficiency results in impaired cell mediated immune responses as measured by *in vitro* lymphocyte function tests and by *in vivo* skin test responses. The clinical data also suggest that iron deficiency may increase the prevalence of common childhood respiratory and intestinal infections to some extent, and that this increase is reversed by iron therapy. However, these epidemiological data are weak and not entirely convincing. There are also data supporting the contention that iron-excess states increase susceptibility to certain pathogens, such as *Salmonella* and *Yersinia enterocolitica*. However, this susceptibility may be mediated by iron dependent or independent mechanisms as discussed. No general state of hypersusceptibility has been convincingly demonstrated thus far. What seems clear is that these data are overinterpreted.

There are few plausible data to support the benefits of iron deficiency on host resistance to infection. What remains debatable is the proper strategy for treating this problem. There seems to be little clinical justification for parenteral iron therapy, especially in young infants, and a safe position is not to recommend oral iron in all circumstances unless a specific medical indication for parenteral iron is present.

If iron deficiency truly represents a clinically immunosuppressive state, then "opportunistic infections" might occur frequently in patients with iron deficiency. One situation supporting this is a reported association between mucocutaneous candidiasis and iron deficiency states. These infections are said to respond favorably to iron administration by some investigators (84,85) but not by others (86,87). The recent association of HIV infection and oral candidiasis as an early sign of AIDS (88) indicates that local *Candida albicans* infection can be opportunistic in the narrow sense of the term. In this context, iron deficiency is not as immunosuppressive as early AIDS, at least as far as this pathogen is concerned. Severe local, disseminated, or recurrent *Herpes simplex* infection is also a problem in immunosuppressed patients. Recurrent *Herpes simplex* response to iron administration has recently been associated with iron deficiency (89). If this is substantiated, it would be the first significant clinical signal that iron deficiency can predispose to opportunistic infection.

In closing, it is worthwhile noting that AIDS in infants is responsible for an increase in otherwise common clinical respiratory and intestinal infections (90). This finding makes it plausible that iron deficiency could similarly result in an increase in such common infections, as already reported in a number of clinical studies on iron deficiency in childhood. It is not necessary to have high grade or severe opportunistic infections to prove that immune mechanisms are being compromised. To the extent that administration of iron could reduce the prevalence of these infections and improve the function of host defenses, morbidity and mortality associated with frequent infections in children in developing countries might be reduced.

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IRON OVERLOAD IN DIFFERENT POPULATION GROUPS

Victor R. Gordeuk,
Department of Medicine
Metropolitan General Hospital
Cleveland, Ohio 44190

Introduction

Although lack of iron is regarded as the most widespread single dietary deficiency around the world (Baker 1978), excess of iron is an important medical condition that achieves the status of a public health problem in some countries. As recently as two decades ago iron overload was thought to be extremely rare (Fairbanks et al, 1971), but now it is recognized that iron loading due to genetic defects or environmental factors is common in certain populations and that parenchymal iron overload is a serious cause of organ dysfunction. Iron overload principally occurs by four mechanisms, the importance of which varies among different population groups. (1) In hereditary hemochromatosis, a genetic defect leads to an abnormal increase in iron absorption (2). In the Thalassemias, ineffective erythropoiesis in the bone marrow is associated with increased absorption of iron from the gastrointestinal tract. (3) With dietary iron overload, excessive iron accumulates from diets with extremely high amounts of bio-available iron. (4) Multiple blood transfusions lead to the inexorable accumulation of excessive iron derived from the hemoglobin of administered red cells.

Iron metabolism

Under normal circumstances, the body's total iron content approximates 45 mg/kg, or about 3000 mg. Of this, two-third or 2000 mg is present in hemoglobin in red blood cells. Ten percent or 350 mg is contained in myoglobin and iron containing enzyme systems. About a fifth of the body's iron, 650 mg, is present as storage iron within cells of the mononuclear-phagocyte system and hepatocytes. The quantity is on the average somewhat more for men than for women. Only about 3 mg of iron circulates in the plasma bound to transferrin. Since the body does not have a mechanism for excreting excess iron, its absorption is carefully regulated so that excessive iron is not acquired. Iron is lost from the body predominantly through the sloughing of cells from the skin, genitourinary tract and gastrointestinal tract. Minute amounts are also excreted in the bile and sweat. For men the daily loss of iron amounts to about 1 mg and precisely the same quantity is absorbed from the diet. In addition to the sloughing of cells, women have blood loss with menstruation which averages, over the month, to approximately 0.5 mg per day. They loose about 1.5 mg of iron from the body daily and absorb the same daily amount from the diet (Bothwell et al, 1979).

Iron absorption in the gastrointestinal tract is controlled by at least three factors, namely the body's iron stores, the amount of dietary iron, and the degree of erythropoiesis. (1) Iron absorption is reciprocally related to the body's iron status. With iron lack there is a stimulus for greater absorption. With increased iron stores, uptake is inhibited (Bezwoda et al, 1919). (2) With increased levels of iron in the gastrointestinal tract, greater amounts of iron are absorbed, but there is careful regulation. Iron may then be incorporated into intracellular ferritin and enter the storage compartment but, under normal conditions, most is released to transferrin in the plasma and transported back to erythroid precursors in the bone marrow (Finch et al, 1970).

Iron overload

Iron overload develops when excessive iron enters the body through abnormally increased absorption or through blood transfusions (Table 1). The absorption of abnormally large quantities of iron may be due to a genetic defect, to ineffective erythropoiesis or to a high dietary content of bioavailable iron. There is then a chronic uncorrectable defect in red blood cell production, and the patient becomes dependent on regular blood transfusions. Infused red cells circulate for a period of time and are then removed by cells of the mononuclear-phagocyte system. Within these cells, hemoglobin is catabolized and iron is freed, but instead of release to plasma transferrin, most of the iron enters the storage compartment. There are two distinct patterns of iron accumulation in iron overload disorders. In one, iron is deposited primarily in tissue macrophages, cells of the mononuclear-phagocytes system. In the other, iron preferentially accumulates in hepatocytes and other parenchymal cells. Tissue macrophages, the predominant site of storage iron under normal conditions, have the capacity to store substantial quantities of iron in the presumably non-toxic forms of ferritin and hemosiderin. Parenchymal cells contain relatively low quantities of iron under physiologic conditions with a limited capacity to hold iron safely, and even with hepatocytes the storage capability can be overwhelmed. Thus, a quantity of iron that might be nontoxic in storage form in cells of the mononuclear-phagocyte system could cause severe toxicity and even death if it were present in parenchymal cells (Table 2) (Finch 1982; Gordeuk et al, 1987). There are two typical conditions: (1) excess iron that is administered in the form of blood transfusions appears predominantly in tissue macrophages; (2) the increased iron absorbed from the gastrointestinal tract in hereditary hemochromatosis is predominantly deposited in parenchymal cells, particularly hepatocytes. It is important to note that as the iron burden becomes massive, redistribution occurs with parenchymal cells and tissue macrophages being affected in both of these disorders. In several conditions, iron loading seems to involve both cells of the mononuclear-phagocyte system and parenchymal cells such as hepatocytes throughout the clinical course. These include iron overload, thalassemias and other hyperplastic refractory anemias (Finch, 1982; Crosby, 1982; McLaren, 1989).

Table 1

I. Increased iron absorption
   A. From normal diets
      1. Hereditary hemochromatosis
      2. Thalassemias
      3. Other hyperplastic refractory anemias
   B. From diets with increased amounts of bioavailable iron
      1. African dietary iron overload
II. Transfusional iron overload
   A. Hypoplastic refractory anemias

When the body's iron burden exceeds the capacity for safe storage, the result is widespread damage to the liver, heart, pancreas and other organs. The specific pathophysiologic mechanisms underlying these toxic effects are poorly understood, but several hypotheses have been offered (Table 3). Iron overload might cause lysosomal fragility with the leak of hydrolytic enzymes into the cytosol leading to damage of subcellular organelles, and, ultimately to cell death (Mak et al, 1985). Iron-induced peroxidation of mitochondrial and microsomal membrane lipids could also lead to cell injury and cell death (Bacon et al, 1985; 1986). Excessive iron may stimulate collagen biosynthesis leading to fibrosis in the liver (Weintraub et al, 1985). Nontransferrin bound iron which may appear in the plasma in all iron overload conditions may also be a source of tissue damage (Hershko and Peto, 1987).

Body iron stores in iron overload can be estimated by indirect and direct measurements. The serum iron and transferrin saturation are usually elevated while the serum

transferrin concentration, often measured by the total iron binding capacity, is in the low or low normal range. The serum ferritin concentration typically is increased. The reference method for measuring the body's iron stores is to perform a liver biopsy and then directly estimate the iron content by staining with Prussian Blue or preferably by measuring the iron concentration chemically (Brittenham et al, 1981). Recently, a non-invasive method for accurately measuring liver iron concentration magnetically has been developed (Brittenham et al, 1982; Brittenham 1988).

Iron overload in different populations

The most common forms of iron overload around the world are due to genetic defects or increased dietary iron. The hereditary forms, namely, hereditary hemochromatosis and the thalassemias, have autosomal recessive inheritance with the heterozygotes displaying little or no iron loading and the homozygotes (or compound heterozygotes) having full disease expression. Iron overload in Africa is clearly related to increased bioavailable iron in the diet, but whether there is also a hereditary component has not been determined. Rare refractory anemias, which can be categorized as hyperplastic or hypoplastic, are found sporadically in all populations and may be associated with iron overload. These conditions, now to be discussed in more detail, are summarized in Table 2.

Table 3

HYPOTHESIZED MECHANISMS OF TISSUE INJURY IN IRON OVERLOAD

I. Postulated Mechanisms
   A. Increased lysosomal fragility
   B. Lipid peroxidation of organelle membranes
   C. Stimulation of collagen synthesis

II. Effects on Tissues
   A. Organelle dysfunction
   B. Cell death
   C. Fibrosis and cirrhosis (liver)

Hereditary Hemochromatosis

Hereditary hemochromatosis, the most common form of iron overload in the United States, has an autosomal recessive mode of inheritance. Although the gene for hereditary hemochromatosis is known to be linked to the HLA locus on the short arm of chromosome 6 (Simon et al, 1977), the precise location of the gene and its normal product

TABLE 2

IRON OVERLOAD IN DIFFERENT POPULATIONS

Condition	Geography	Predominant Age Group	Estimated Prevalence in Populations at risk	Mechanism of Iron Loading	Early Site of Excess Iron	Approximate Lethal Iron Level (mg/kg body weight)	Therapy
Hereditary Hemochromatosis	Europe	Adults	4.5/1000	Increased iron absorption	Hepatocytes	200 - 400	Phlebotomy
Dietary Iron Overload	Sub-Saharan Africa	Adults	12/100**	Increased Dietary Iron	Mononuclear-phagocyte cells and hepatocytes	400 - 1000	Phlebotomy
Thalassemias*	Asia, Middle East and Mediterranean	Children	>100,000 affected infants born/year	Ineffective erythropoiesis and transfusions	Mononuclear-phagocyte cells and hepatocytes	400 - 1000	Iron Chelation
Hypoplastic Refractory Anemias	Worldwide	Adults and Children	Rare	Transfusions	Mononuclear-phagocyte cells	1000-2000	Iron Chelation

* - Other rare hyperplastic refractory anemias have similar mechanism and site of excess iron; distribution is worldwide.

** - Adult males who consume traditional beer.

have not been identified yet. An association of the condition with the HLA-A3 antigen has been described (Simon 1985).

In homozygotes for hereditary hemochromatosis, iron absorption is inappropriately high for any amount of storage iron. Iron loading first affects hepatocytes (Edwards et al, 1977) and then the parenchymal cells of other issues. Cells of the mononuclear-phagocyte system, the predominant location of storage iron under physiologic circumstances, may have normal or even decreased storage iron in the early stages of the disease (Valberg et al 1975). A chronic positive iron balance occurs in homozygotes for hemochromatosis and this may result in damage to the liver, heart, endocrine organs, joints and other tissues (Niederau et al 1985). Symptomatic expression of the condition is usually delayed until middle or late life when body iron burden reaches 100 to 200 mg per kg body weight (Finch and Heuber, 1982). Mortality and morbidity from hereditary hemochromatosis appear to be preventable if patients are diagnosed and excess body iron is removed before irreversible liver damage occurs (Niederau et al, 1985). Heterozygotes for hereditary hemochromatosis may have a mild degree of iron overload but that has not been regarded as a cause of adverse pathologic consequences (Cartwright et al, 1979). A recent report raises the possibility that the heterozygous state may increase the risk for certain forms of cancer. Participants in the National Health and Nutrition Survey who later developed carcinoma of the bladder, esophagus and lung had moderate elevations in mean transferrin saturations (Stevens et al, 1988) similar to values found in people who are heterozygous for hereditary hemochromatosis (Borwein et al, 1983).

The prevalence of hemochromatosis has been studied most thoroughly in Europe and in populations largely derived from Europe such as Australia and the United States. Its incidence in other parts of the world and other population groups is not known (Whittaker et al, 1989). A few decades ago it was thought that hereditary hemochromatosis is an extremely rare condition with an occurrence of about 0.5 in 10,000 (Fairbanks et al, 1971). More recent surveys have estimated the prevalence of homozygotes to be 2.5 to 5.0 per 10,000. A study of 11,065 Utah blood donors reported in 1988 revealed a gene frequency of .067 and a prevalence of homozygotes of 4.5 in 1000. It was projected that 12.5% of the population are heterozygous for hereditary hemochromatosis (Edwards et al, 1988). It has been postulated that in the past, the hemochromatosis trait has provided a selective survival advantage for individuals with the gene, particularly women of child bearing age in whom the slight increase in iron absorption associated with the heterozygous state may have served to prevent the occurrence, or decrease the severity, of iron deficiency anemia.

Manifestations of hereditary hemochromatosis include liver fibrosis and cirrhosis, congestive heart failure, pancreatic failure, arthritis, and sexual dysfunction. In untreated patients death commonly occurs due to hepatocellular carcinoma, cirrhosis, cardiomyopathy or diabetes mellitus (Niederau et al, 1985). The disease can be effectively treated by removing iron by phlebotomy. Typically, such a program involves the removal of one or two units of blood a week for one or more years until the excessive iron has been removed from the body as manifested by a state of iron deficiency. One then continues to remove one unit of blood every one to two months to prevent reaccumulation of iron. In patients who are effectively treated with phlebotomy before cirrhosis of the liver develops, the life span is normal and there is no increased incidence of hepatoma. Once cirrhosis has developed, liver fibrosis is irreversible and the patient has a high risk of the development of hepatocellular carcinoma despite vigorous phlebotomy therapy (Niederau et al, 1985). The congestive heart failure that occurs with hereditary hemochromatosis responds well to phlebotomy therapy (Bothwell et al, 1979).

Thalassemia

The thalassemias are part of a group of refractory iron loading anemias characterized by hypercellular marrows and erythroid hyperplasia in which massive iron overload may develop that is not accounted for by red cell transfusions. In the thalassemias there is defective production of one of the globin chains which are components of the hemoglobin molecule. Beta thalassemia is characterized by reduced beta globin synthesis and alpha thalassemia by decreased alpha globin production. The beta globin gene is normally located on chromosome 11 in single copy and the alpha globin gene is present in duplicate on chromosome 16. A normal red cell precursor has two functioning beta globin genes and four working alpha globin genes. Thalassemia minor refers to the conditions in which only one beta globin gene or just two alpha globin genes function normally. In these conditions the hemoglobin is in the low normal or slightly anemic range and microcytosis is present but there are few or no pathophysiologic consequences. Iron overload does not occur. Beta thalassemia major and intermedia refer to conditions in which both beta globin genes are defective. With beta thalassemia major, there is absent production of beta globin and with intermedia, it is produced at a markedly reduced rate. Hemoglobin H disease is the condition in which three of the four alpha globin genes are deleted or not functioning. Beta thalassemia major and intermedia as well as hemoglobin H disease are characterized by moderate to severe hemolytic anemia and splenomegaly (Jandl, 1987). There is marked ineffective erythropoiesis with an associated stimulus for increased iron absorption. Iron loading begins early in childhood and can become massive even in the absence of blood transfusions (Bothwell et al 1979; Pootrakul et al 1989). In addition, a variety of hemoglobinopathies such as hemoglobin E produce the phenotype of thalassemia major or intermedia when present with one beta thalassemia gene (Pootrakul et al, 1988).

The distribution of thalassemias around the world is very similar to the historic endemicity of malaria. This observation has led to the idea that the thalassemia trait may be protective from malaria and that there has been a selective survival advantage for affected individuals in populations that inhabit areas where malaria infection has been prevalent (Nagel and Ruth, 1989). Several lines of evidence support this hypothesis. (1) Impaired growth of malaria parasites in vitro in erythrocytes from patients with thalassemia has been demonstrated (Brockelman et al, 1987). (2) In several populations, an increasing frequency of the beta thalassemia trait with greater age has been found. This is compatible with the possibility that children who are the most vulnerable to severe malaria infection have improved survival if they express the thalassemia trait (Wilcox et al, 1983). (3) In children 1 to 4 years of age, the probability of lethality of malaria infection as estimated by parasite density was calculated to be .45 in subjects with thalassemia trait as compared to individuals without the trait (Wilcox et al, 1983).

In the thalassemias, intestinal iron absorption may be increased as much as ten-fold above normal and iron loading begins in early childhood. The excess iron is deposited both in cells of the mononuclear-phagocyte system and in parenchymal cells. Initially, hepatocytes are affected but eventually the pancreas, heart and other organs are also involved. The magnitude of iron overload can be similar to that found in hereditary hemochromatosis (Pootrakul et al, 1988). In these patients, iron overload cannot be managed with phlebotomy therapy as it is in hereditary hemochromatosis because of the presence of anemia, and treatment with an iron chelating agent capable of binding to iron and facilitating its excretion from the body must be used. Desferrioxamine, a naturally occurring trihydroxamic acid produced by *Streptomyces pilosus*, is the only drug currently approved for iron chelation therapy. Its absorption from the gastrointestinal tract is poor, and to be effective, the drug must be given by subcutaneous or intravenous infu-

sion, ideally for twelve hours a day. Iron chelation therapy with desferrioxamine can prevent organ damage and improve survival in patients with thalassemia major (Brittenham, 1987).

The thalassemias are most prevalent in countries bordering the Mediterranean Sea, in the Middle East and in central and southern Asia (Weatherall et al, 1989). On the basis of the proportion of heterozygotes in the population, the prevalence of beta thalassemia major at birth can be estimated to be approximately 3 in 1000 in parts of Italy and Greece (Tentori and Marinucci, 1986; Loukopolos 1986), and the condition is an important health problem from Northern Africa to Southeast Asia. Although alpha and beta thalassemia traits have similar geographic distributions, hemoglobin H disease is a more localized health problem than beta thalassemia major. The reason is that for hemoglobin H disease to occur, both alpha-1 trait, in which two alpha genes are deleted from a single chromosome, and alpha-2 trait, in which only one alpha gene is deleted from a particular chromosome, must be present in the same population. This occurs most commonly in Southeast Asia, where the prevalence at birth of hemoglobin H disease may achieve about 2 in 1000 in Thailand, and the Mediterranean islands (Wasi 1983; Weatherall et al, 1989). It has been estimated that over 100,000 children per year are born with thalassemic syndromes that are associated with iron loading (WHO Working Group 1983).

Other hyperplastic refractory anemias

In addition to the thalassemias there are other rare hyperplastic refractory anemias that are associated with increased iron absorption and that, for the most part, have a worldwide sporadic occurrence. These disorders include sideroblastic anemias, congenital dyserythropoietic anemias, pyruvate kinase deficiency and a number of anemias associated with blocks in the incorporation of iron into hemoglobin (Bothwell et al, 1979). The pattern and consequences of excessive iron and the need for iron chelation therapy in these conditions are similar to the thalassemias (Pootrakul et al, 1988).

Transfusional iron overload

Patients with a variety of hypoplastic refractory anemias develop iron overload as a consequence of repeated blood transfusions. These disorders include the congenital Blackfan-Diamond syndrome and anemias that appear later in life such as aplastic anemia, pure red cell aplasia, myelodysplastic disorders and anemia of chronic renal failure. Each unit of transfused red cells contains 200 - 250 mg of iron. The body does not have a mechanism for excreting this iron load and with a chronic transfusion program, an excess of iron gradually builds up. In transfusional iron overload, the excessive iron initially accumulates in the relatively benign mononuclear-phagocyte sites. As massive iron stores build up, redistribution to parenchymal sites occurs. A given level of excessive iron tends to be less toxic in patients with transfusion-dependent anemia as compared to patients with hemochromatosis and iron loading anemias with ineffective erythropoiesis. Eventually, however, cardiac dysfunction, liver disease and endocrine disorders develop, and in untreated patients, death typically occurs as a result of heart disease (Schafer et al, 1981). In patients with thalassemia major and intermedia on a high transfusion regimen, erythropoiesis is suppressed and iron overload occurs because of the ongoing accumulation of iron with the transfusion regimen (Modell and Beroukas, 1984). By definition, patients with transfusion-dependent anemias cannot be managed with a phlebotomy program to remove excess iron. The only approach available at the present time is parenteral desferrioxamine therapy.

Dietary iron overload

Sub-Saharan Africa is the only part of the world where iron overload has been clearly documented to result from increased iron in the diet. It was first described in South

African blacks in 1929 and has since been reported in a number of other countries including Botswana, Kenya, Malawi, Mozambique, Swaziland, Tanzania, Zambia, Zimbabwe, and Lesotho. In these populations, excess dietary iron is derived from the traditional beer that is home- brewed in steel drums from locally grown crops. The iron is in an ionized highly bio-available form. Although it was reported that iron overload has been decreasing in urban dwellers who are becoming more affluent and are consuming commercial rather than traditional beer (MacPhail et al, 1979), a recent survey indicates that dietary iron overload remains a contemporary public health issue in rural populations of sub-Saharan Africa. In a rural Zimbabwe community, 68% of men gave a history of the consumption of traditional beer. Seventeen percent of these men had a high serum ferritin and a transferrin saturation of over 70%, a combination that indicates a risk of liver disease and other pathologic effects from excess body iron (Gordeuk et al, 1986). If these findings are applied to the general population of Zimbabwe, the number of men with an iron overload of sufficient severity to cause toxic manifestations could be over 100,000.

In African dietary iron overload, excessive iron accumulates in both cells of the mononuclear-phagocyte system and in tissue parenchymal cells, particularly the hepatocytes (Brink et al, 1976). Clinically, the condition usually presents as hepatic portal fibrosis and cirrhosis, but it also seems to be related to the appearance of scurvy, osteoporosis, diabetes mellitus, idiopathic heart failure, esophageal carcinoma and infections (Bothwell et al, 1979). Although phlebotomy therapy would be expected to be beneficial, the efficacy of venesection has not been adequately examined. A single preliminary study from Tanzania reported encouraging results (Clift and Speight 1976). Iron overload from dietary means is a preventable disease. There are needs for educating the populace on the dangers of consuming iron-laden beer and working with communities in developing culturally acceptable methods to produce traditional beer with low iron concentration.

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MALNUTRITION, WORK PERFORMANCE AND THE ROLE OF IRON

G. B. Spurr
Department of Physiology
Medical College of Wisconsin and
The Research Service
Zablocki VA Medical Center
Milwaukee, WI USA
and
Department of Physiological Sciences
Universidad del Valle
Cali, Colombia

The single most prevalent nutritional deficiency in both developing and developed countries is that of iron intake with its concomitant iron deficiency anemia (1,2). Chronic energy deficiency (CED) is also a widespread phenomenon, particularly in the less developed world where nutritional (iron deficiency) anemia is strongly associated with nutritional status (3). In addition to CED, with its socioeconomic implications (2), pregnant women [(4) p228] and athletes (5) also seem to be at increased risk of iron deficiency. Whatever the reason, iron deficiency (ID) and anemia have been shown to have effects on physical work capacity and performance. It is the purpose of this paper to attempt to describe the separate effects of ID, anemia and chronic malnutrition on work capacity, endurance and performance in physical exercise.

While the interrelationship between iron stores, hemoglobin, myoglobin and the cytochromes have been determined for the rat under conditions of ID which do not interfere with growth (6). Similar measurements do not seem to have been made in humans. However,three stages of ID are recognized. The first occurs when iron stores are substantially reduced. These stores amount to about 1000 mg in the adult male and 300 mg in the adult menstruating female (7). Serum or plasma ferritin is a useful index of body iron stores and is considered abnormal and indicative of decreased iron stores when below about 12 ug/L. The second stage occurs in the period between the depletion of iron stores and the beginning of frank anemia, and is characterized by decreased transferrin saturation and increased erythrocyte protoporphyrin. The third phase of ID is the most severe and results in microcytic and hypochromic anemia. This stage is identified by decreased hematocrit and hemoglobin concentration and reduced mean corpuscular volume.

The effects of ID on the oxygen carrying capacity of the arterial blood in the latter stage have been the most studied of these three phases. But beyond its important role in the synthesis of hemoglobin (Hb), iron is necessary for the proper function of practically all tissues because it is involved in a large number of energy producing systems including the mitochondria, cytochrome system and flavoprotein. It is these enzyme systems which may be the basis for the direct effects of ID on performance and work capacity. Iron is also a component of myoglobin.

The physical fitness of an individual is best estimated by the maximal work capacity (physical work capacity; PWC) as measured

by the maximum oxygen consumption (VO_2 max). The PWC or VO_2 max is the result of a number of contributing factors, the more important of which include genetic endowment, physical condition (training), sex, age and nutritional status. The VO_2 max is usually measured on a treadmill or bicycle ergometer. The methods employed and the factors which affect it have been described (8).

Another important component of work performance is endurance which, in the present context, means the maximal time an individual can work or exercise at a given submaximal workload.

The role of iron and malnutrition (undernutrition) on work performance will be discussed in terms of reduced oxygen carrying capacity of arterial blood (anemia), the direct effects of ID on work capacity and performance, and finally, the results of nutritional compromise. No attempt is made to be all inclusive in reviewing the literature but rather to select those results which best describe the underlying physiological relationships as they are currently understood.

Anemia

There is general agreement that acute and chronic reductions in blood Hb levels reduce work capacity and endurance. The effect of anemia on work performance is related to the balance among the severity of the reduced Hb concentration, the level of work employed in the testing procedure (maximal vs submaximal) and the ability of the subjects to endure given submaximal work loads.

Sproule el al (9) seem to have been the first to attempt the measurement of VO_2 max in anemic subjects (mean Hb=7.2 g/dl). However, their mean values for nine patients include three subjects who do not reach VO_2 max and so the comparisons with normal subjects are biased towards low values for those with low Hb. They did report that their anemic subjects achieved relatively high work loads (average=6 mph and 12.5% grade) without any more serious symptomatic stress than that observed in the non-anemic subjects.(9)

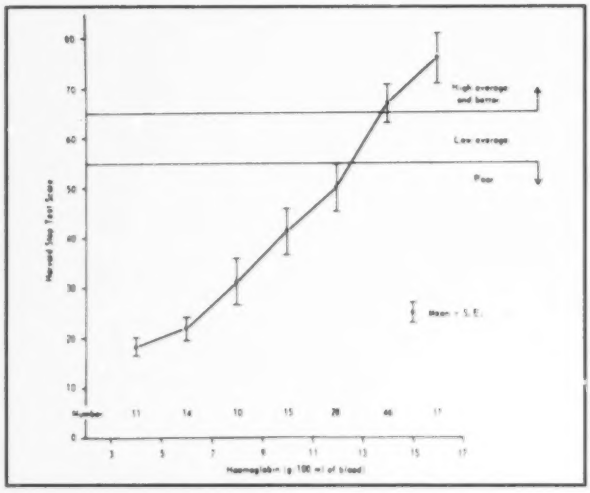

Figure 1—Harvard Step Test (HST) score for different hemoglobin concentrations in agricultural labourers in the lowlands of Guatemala (From Viteri and Torun (10)).

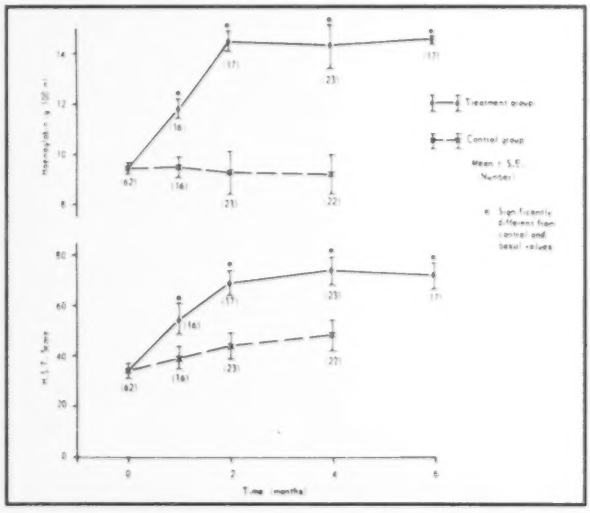

Figure 2—Changes in hemoglobin concentration and in physical fitness (HST score) in a control and a treated population of anemic adult men in the lowlands of Guatemala. Treatment consisted of 100 mg of ferrous sulphate daily and 5 mg of folic acid weekly. (From Viteri and Torun (10)).

Perhaps, the most widely quoted work showing the effect of anemia on work capacity is that of Viteri and Torun (10) on agricultural laborers in Guatemala. They employed

the Harvard Step Test (HST), which is related to VO_2 max (11) to show decreases in the best HST scores with relatively small decreases in blood Hb and a marked improvement following treatment with iron and folic acid. These results are shown in Figures 1 and 2. Similar results have been found in industrial workers in Africa (12), in rubber tappers in Indonesia (13) and tea pickers in Sri Lanka (14-16) where, in addition to the impaired work performance in anemia and recovery with improved Hb following treatment with iron, work productivity is found to be positively related to the blood Hb levels. The relationship between blood Hb and work capacity and endurance has also been amply confirmed in experiments on rats (17-19). In addition, in both rats (20) and adult humans (16), anemia results in decreased voluntary activity. On the other hand, Gandra and Bradfield (21) are unable to detect any significant difference in activity levels of normal (Hb >12g/dl) or anemic (Hb <10g/dl) children (grades 1-3) in Brazil before or after treatment with iron of some of the anemic subjects. However, the authors thought that perhaps physical and cultural factors might have played a more important role in their study than blood Hb levels.

The effect of anemia on the work capacity of school-aged children is not as clear as that seen in rats or adult humans. We have measured the VO_2 max in 1,010 normal and marginally undernourished boys in Colombia (22). The results are shown in Figure 3 as a function of average blood hemoglobin levels between 9.4 and 15.7 g/dl. There was no significant correlation between the two variables. Vellar and Hermansen (24) have reported similar results in children although their blood Hb values did not fall as low as seen in Figure 3. On the other hand, Davies (25) found reduced work capacity (VO_2 max in ml/min/kg) in 14 year old boys with average blood Hb of 8.0 g/dl. Parsons and Wright (26), after measuring the heart rate responses to stair climbing in anemic children, stated that "with chronic anemia, adaptation to the low hemoglobin concentration may be so complete that practically no impairment of exercise tolerance results." These observations and those presented in Figure 3 point to possible physiological adaptations in children during chronic anemia which deserve further study.

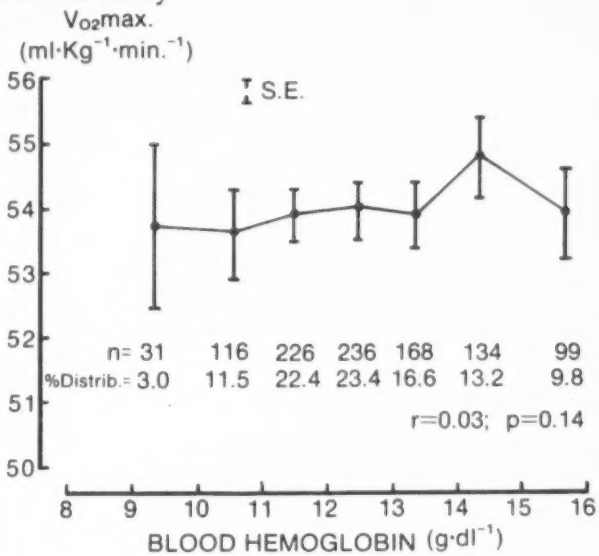

Figure 3. Average (± SE) O2 max (ml/min/kg) as a function of average blood hemoglobin in normal and marginally malnourished boys (From Spurr (23)).

Celsing et al (27) have investigated the responses of the central circulation to provide some understanding of the physiological adaptation to submaximal and maximal exercise as a result of chronic anemia induced for a period of 8-11 weeks. Measurements were made prior to the induction of anemia, after about 9 weeks of anemia induced and sustained by repeated venesection in 9 healthy subjects, and 48 hours after re-transfusion of the subjects' stored blood. The responses of some parameters for the submaximal and maximal exercise tests are shown in Figures 4 and 5. The VO_2 for the submaximal bicycle exercise (150 or 175 W) was not affected by the anemia (Figure 4). It was maintained by an increased cardiac output which was mediated principally by an increased heart rate without significant change in stroke volume (Figure 4). The significant de-

crease in systemic O_2 transport that can be calculated from cardiac output and VO_2, was made up by the greater extraction of oxygen from the arterial blood reflected in the increased fraction of O_2 extraction (Figure 4).

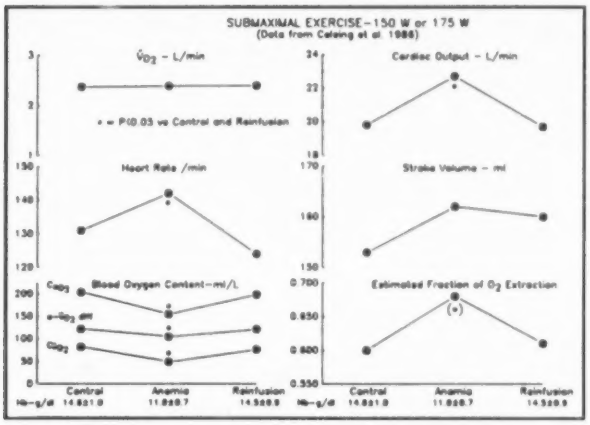
SUBMAXIMAL EXERCISE – 150 W or 175 W
(Data from Celsing et al. 1986)
V̇O2 – L/min
Cardiac Output – L/min
* = P<0.05 vs Control and Reinfusion
Heart Rate /min
Stroke Volume – ml
Blood Oxygen Content – ml/L
Estimated Fraction of O2 Extraction
Control
Anemia
Reinfusion

Figure 4. Mean values of several variables during submaximal bicycle exercise before, during and after 8-11 weeks of anemia produced by repeated venesection (Data from Celsing et al. (27)).

In the case of VO_2 max, Celsing et al (27) reported a marked reduction in VO_2 max (Figure 5) which has been a common finding in both acute (28-30) and chronic (9,10,12) reductions in the oxygen carrying capacity of arterial blood. This would seem to be due to the reduced maximal cardiac output resulting largely from reduced maximum heart rate since stroke volume, although diminished, was not significantly changed (Figure 5). The reduced maximum cardiac output, decreased O_2 carrying capacity of the arterial blood and reduced a-v O_2 difference lead to lower VO_2 max values despite lower mixed venous O_2 content and increased extraction fraction (Figure 5). The reason for the decreased maximal cardiac output at the end of the anemic period and before reinfusion,was not clear to the authors (27). Three months following the reinfusion, maximum heart rate values had returned to control values. In many respects the results of both submaximal and maximal exercise testing obtained by Celsing et al (27) were similar to the findings of others (9,30). The increased extraction of O_2 (Figures 4 and 5) is certainly at least partially due to the increased erythrocyte concentration of 2, 3 diphosphoglycerate (2,3 DPG) found in anemia (30). However, a detailed understanding of the mechanisms of adaptation to chronic anemia awaits further experimentation.

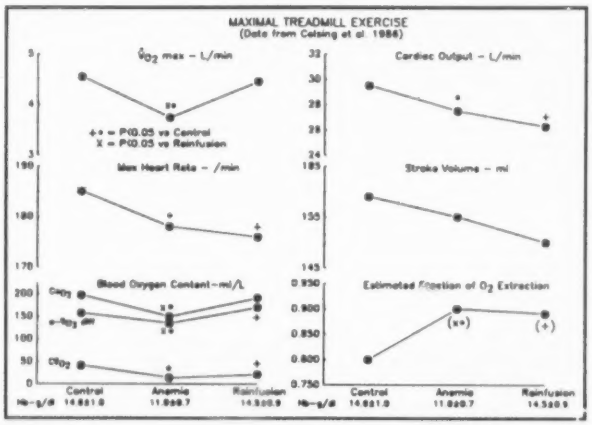

Figure 5. Mean values of several variables in response to maximal treadmill exercise before, during and after 8-11 weeks of anemia produced by repeated venesection (Data from Celsing et al. (27)).

Iron Deficiency Alone

Severe, chronic ID will always end up in anemia with the consequences for work capacity already discussed. Consequently, those experiments which attempt to reveal the direct effects of ID alone require the use of artificial manipulation of the system under study. This will usually involve some variation of repeated venesection to induce ID and anemia, with correction of the latter by infusion of packed red cells or of iron supplementation which does not affect blood Hb.

Perhaps the earliest recognition of the possibility that iron might have an effect on work capacity independently of blood Hb was that of Ericsson (31) in 1970. He administered 120 mg of ferrous fumarate daily to healthy, non-anemic, elderly men and women and compared their PWC's at the

end of 3 months to those measured in a placebo group. He concluded that the increase in physical work capacity, measured during a submaximal bicycle ergometer test, was probably related to the availability of iron (31).

Finch et al. (32) studied iron deficient rats with normal Hb concentration and found a marked impairment of running ability compared with control groups of animals. The impairment was corrected within 4 days by treatment with iron. Concentrations of the cytochrome pigments and myoglobin and rates of oxidative phosphorylation with pyruvate-malate, succinate and alpha-glycerophosphate substrates were all decreased in skeletal muscle mitochondrial preparations. In work performed by the iron deficient rats which were treated with iron, only the rate of phosphorylation with alpha-glycerophosphate as substrate increased significantly and in parallel with the work performance recovery (32). Subsequent experiments on lactic acidosis in iron deficient rats during running seemed to confirm that the reduced performance of these animals was due to elevation of blood lactate resulting from depletion of muscle mitochondrial alpha-glycerophosphate oxidase which in turn impairs glycolysis (33).

Detailed studies by Davies et al. (34, 35) have further delineated specific aspects of muscle function in chronic ID of rats. They induced severe iron deficiency by feeding weanling rats an iron deficient (2 mg/day) diet or a control (50 mg/day) diet for 22 days. The ID rats developed severe anemia (Hb < 4 g/dl; hematocrit < 20%) as well as depletion of muscle myoglobin and cytochrome c relative to controls. At 42 days of age (day 0 of the study), the ID rats were switched to the control diet and entered the phase of iron repletion for the subsequent 7 days. There was a rapid return of Hb and hematocrit towards the values observed in the control animals and of the values for cytochrome oxidase, pyruvate-malate oxidase, flavoprotein, cytochrome a and c enzymes. There was also a return of the originally depressed (day 0) VO_2 max and endurance times towards control values (Figure 6). The former more closely followed the pattern of correction in the anemia, as evidenced by the hematocrit, and the endurance, the correction of the deficiency in muscle oxidative enzymes, represented by the pyruvate-oxidase system (Fig. 6) (34). The 50% initial reduction in work capacity was increased on day 3 of iron repletion while endurance capacity did not improve significantly until day 5, and there was no significant increase in muscle energetic capacities before day 5. It seemed unlikely therefore, that the muscle enzyme systems were limiting to the work capacity but rather that endurance was limited by oxidative capacity of the muscles at submaximal levels of effort while the high level maximum effort was restricted by hemoglobin and the oxygen carrying capacity of blood (34). That is, that they had been able to show a separation of endurance from maximum working capacity.

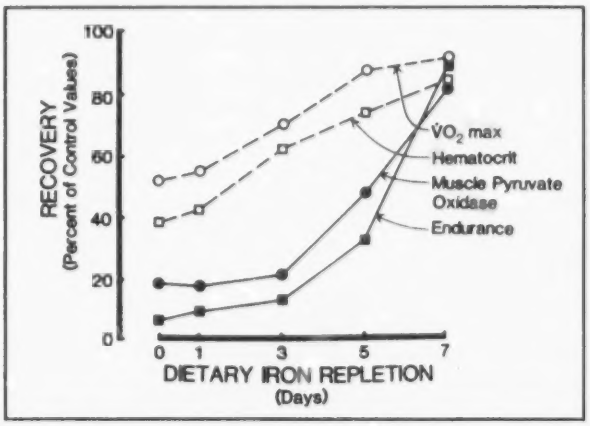

Figure 6. Recovery of VO_2 max, hematocrit, muscle homogenate pyruvate-malate oxidase activity and endurance capacity during dietary iron repletion. Values are reported for iron-deficient rats on each day of study and are expressed as percentages of each day's control group results (From Davies et al (34)).

This same group of investigators then conducted a series of experiments on similar ID and control rats in which the VO_2 max and the endurance times were measured in ID (Hb =

3.9 g/dl) and control rats (Hb = 14.2 g/dl) and in similar animals whose blood Hb had been equalized (about 9-10 g/dl) by exchange transfusion of either plasma to lower blood Hb of control animals or packed red cells to increase the Hb of the rats made anemic by iron deficiency. The results are shown in Figure 7. In the control animals there were no differences between the sham and plasma perfused groups in VO_2 max, treadmill speed atVO_2 max or maximal endurance time. The iron deficient animals exhibited significant reductions in all three parameters. Elevation of the blood Hb increased both VO_2 max and maximum treadmill speed to almost control levels but the endurance time was unaffected, again pointing towards the primary dependence of endurance on muscle enzymes and adding to the evidence for separation of the dependence of endurance from VO_2 max in this species. In any event, it would seem clear

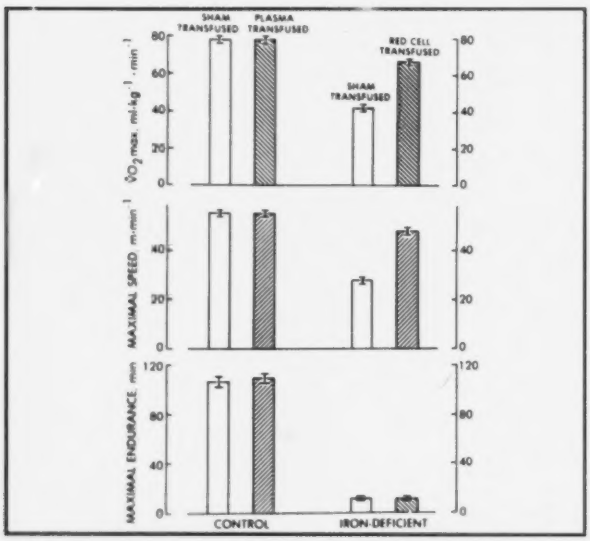

Figure 7. Endurance capacity, VO_2 max, and VO_2 max work load (maximal speed) of transfused and sham-transfused, iron-deficient and control rats (n=6 per group). Endurance capacity was measured with an endurance test (20.1 m/min and 15% grade) and VO_2 max and VO_2 max work load were measured in the separate progressive-intensity test (From Davies et al (35)).

that the effects of anemia and of ID alone are distinct in the rat, with the former primarily affecting physical work capacity while the latter predominantly affects the ability to endure a submaximal work load.

The situation in humans is not as clear. Celsing and co-workers have measured muscle enzymes in acute (about 4 wks), experimentally induced ID produced by repeated venesection (36) and in the chronic ID caused by long term intestinal bleeding or repeated pregnancy combined with low iron intake found in a population of Indonesian volunteer subjects (37). They were unable to demonstrate significant changes from control in glycolytic, oxidative and iron dependent enzymes of skeletal muscle. In the experimentally induced ID it was possible to correct the associated anemia by transfusion of packed red cells and thereby separate the effects of ID and anemia on VO_2 max and endurance. The data are summarized in Figure 8 which shows the results of the iron status, Hb concentration of arterial blood, VO_2 max and endurance times in the control, anemic and post-transfusion periods of the study. In anemia, 8-11 weeks after beginning the venesections (about 4 weeks of stable anemia), there were significant reductions in all the parameters. Following correction of the anemia by reinfusion of blood, both VO_2 max and endurance time returned to control values while the ID was not affected. These results do not agree with those found in the rat (34,35) and may be related to the less severe degree of anemia and ID in the experiments on humans than in those on rats. However, the levels produced in the human studies (36) are those which might be found in at-risk populations (37). Also, the results reported in rats may not be strictly extrapolatable to humans (36).

Two additional reports are relevant to the work just described. Rowland et al. (38) described experiments in which 14 iron-deficient (serum ferritin < 20 ug/l), non-anemic female cross country runners were measured for their exercise capacity before and after

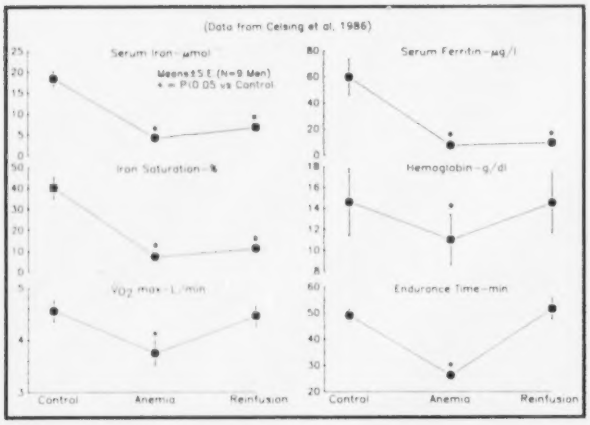

Figure 8. Means ± S.E of iron status, blood hemoglobin, VO_2 max and endurance time in adult male human subjects. Where S.E's do not appear they are covered by the mean value. (Data from Celsing et al (37)).g

one month of treatment with ferrous sulfate in one half of them. While serum ferritin rose significantly in the treated subjects (8.7 to 26.6 ug/l), it fell slightly in the placebo group. There was no significant change in either group in VO_2 max or in submaximal VO_2. They reported a significant improvement in "endurance time" in the treated compared to the placebo groups (38). However, the endurance times they reported were those times it took to reach maximum VO_2 values on a fixed treadmill protocol. Most treadmill protocols are designed to achieve maximum VO_2 in a minimum time or at least before fatigue occurs. The "endurance times" reported by them of 16-17 minutes are probably not related to the true endurance times achieved while running at 80-90% VO_2 max (36). Consequently, these studies (38) are not contradictory to those of Celsing et al. (36). Newhouse et al. (39) also studied the effect of 8 weeks of iron treatment of prelatent/latent iron deficiency (serum ferritin about 12 g/l) on the work capacity and muscle enzymes of female endurance runners. Treatment resulted in increased serum iron values but no significant change in work capacity parameters or muscle enzymes (citrate synthase and cytoplasmic alpha-glycerophosphate dehydrogenase activity). They concluded that serum ferritin below 20 ug/l does not pose a significant handicap to physical work capacity (39). They did not measure endurance times.

To summarize briefly, it would seem that in ID, the experimental rat shows changes in iron dependent muscle enzymes which permit a separation of endurance from VO_2 max such that there is a significant reduction in endurance time which does not affect work capacity (VO_2 max) when the associated anemia is corrected (36). At the levels of ID and anemia usually found in humans, this does not seem to be the case. Both VO_2 max and endurance times are corrected by raising blood Hb levels despite continued indications of ID, and muscle enzymes are not changed. At the moment it is not known whether the apparent conflict is due to differences in intensity of ID and anemia or to the species themselves. We must await the accumulation of further data.

Malnutrition:

Studies of the effects of malnutrition on PWC have been of two kinds. The experimental malnutrition induced by caloric restriction of previously well-nourished subjects under controlled laboratory conditions for periods of a few days to six months or the undernutrition found under free-living conditions where the individual(s) may have been exposed to a losing battle for an "adequate" diet for shorter or longer periods up to the life time of the subject, form the bases for the most common types of studies in humans. While acute and chronic are relative terms, for the purposes of the present discussion, acute studies will be applied to the shorter, laboratory studies and chronic to field studies of individuals living under the deprived economic conditions frequently found in developing countries. The relationship between nutritional status and PWC has been treated in detail (23).

Acute:

Reductions in VO_2 max have been found in studies of complete starvation for periods as short as 4.5 days (40). Perhaps the best known of the experimental studies of semi-starvation are the classical experiments performed at the University of Minnesota during the latter part of World War II (41). Thirty-two volunteer male subjects were studied extensively for several weeks while consuming a diet containing approximately 3500 kcal/day and during a 24 week period on a diet of about 1600 kcal/day. During a 12 week recovery period equal number of subjects ingested diets of 2378, 2692, 2896 and 3123 kcal/day. The Harvard Step Test was performed in all subjects and VO_2 max measured in 8 individuals before semi-starvation and at 12 and 24 weeks of the low calorie diet. The HST measurements were continued at 6 and 12 weeks of the recovery period. There was a progressive depression in VO_2 max at 12 and 24 weeks of semi-starvation when expressed as L/min, ml/min/kg of body weight or ml/min/kg of lean body mass (LBM). Although VO_2 max was not measured directly during the recovery period, return of PWC towards control (as measured by HST) was a slow process, the rate being roughly proportional to caloric content of the recovery diet. The blood Hb levels fell from an average control value of 15.1 g/dl to 12.6 and 11.7 g/dl at 12 and 24 weeks of the low calorie diets. Consequently, a part of the reduced VO_2 max was no doubt due to developing anemia although the loss in muscle mass can be considered as contributing in a major way to the reduced VO_2 max (23). The author is unaware of any studies in which the correction of the anemia associated with malnutrition has been done to measure its contribution to the observed reduction in VO_2 max of undernourished subjects.

Chronic:

Studies of PWC in undernourished subjects have been performed in adult men and on children.

A) Adults:

The earliest studies of PWC in undernourished subjects are those of Viteri (42) and Viteri and Torun (43) on Guatemalan agricultural workers. There were reduced VO_2 max measurements in workers receiving a dietary supplementation, those without supplementation and recent army recruits from the same socioeconomic circumstances as the agricultural workers, when compared to army cadets who had never been exposed to undernutrition (42,43). The authors concluded that the lower values were due to differences in body composition rather than to any fundamental impairment of muscle function (42,43).

We have studied three groups of chronically malnourished adult males selected for their existing degree of malnutrition (44,45) and of the most severely malnourished group during 45 days of basal maintenance and 79 days of dietary repletion on a high protein diet (46, 47). These studies are summarized in Figure 9. On the right are plotted mean values for control (C) subjects and those with moderate (M), intermediate (I) and severe (S) malnutrition based on their weight/height and daily creatinine excretion/height ratios and their serum albumin levels (44). The data for the S group is also shown during a basal period of adequate caloric intake (2240 kcal/day) and the same low protein (27 g/day) ingested prior to entrance into the hospital, followed by dietary repletion with an isocaloric diet containing 100 g/day of protein. The progressive differences of all variables with increasing severity of malnutrition are obvious as are their gradual return towards control values during dietary repletion following a period of no change during the basal diet. Similar results were observed in the measurements of VO_2 max shown in the left panel both when expressed as L/min and ml/min/kg body weight. In these experiments there was a spontaneous separation of the anemia from body composition changes since VO_2 max increased significantly approximately midway through the repletion period prior to Hb

and hematocrit (Fig 9). This would indicate that the increase in VO_2 max at this point was due to a change in some other factor. By comparing the VO_2 max expressed as ml/min/kg of various body compartments in the 3 groups of nutritionally compromised subjects, it was possible to estimate that over approximately 80% of the difference in VO_2 max between M and S groups was due to differences in muscle mass with the remainder possibly being due to differences in the oxygen carrying capacity of the arterial blood of the two groups (23,47).

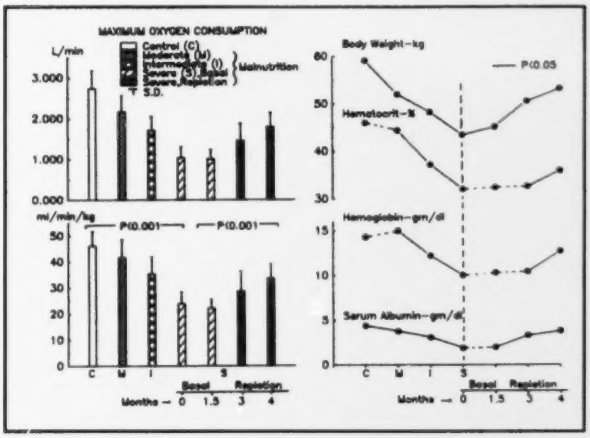

Figure 9. Mean +S.D of VO_2 max as L/min and ml/min/kg body weight on left and means of body weight and blood values on right, of men classified as nutritionally normal (C) or moderate (M), intermediate (I) and severe (S) in the degree of their nutritional compromise. The S group is also shown during 1.5 months of a basal diet of 2240 kcal/day and 27 g/day of protein followed by 2.5 months of an isocaloric diet with 100 g/day of protein. (Data from Barac-Nieto et al (44-47)).

B) Children

We have done extensive studies on the effects of chronic marginal malnutrition on the growth and physical work capacity in Colombian school children 6-16 years of age (48,49). The VO_2 max (L/min) was lower in undernourished children than in their nutritionally normal counterparts throughout the age range studied (48,49) and was lower in girls than in boys (49). The differences in VO_2 max due to the effect of nutritional status are largely the result of differences in body size in the two groups as can be seen in Figure 10 where the VO_2 max is plotted as a function of body weight and of LBM. The average values of six age and nutritional groups of boys all fall on the same straight lines as do the values for girls. The fact that the data for girls fall on a different straight line than the boys is a reflection of lower VO_2 max values in girls even when the differences in body composition due to sex are removed in the plots on LBM (Fig 10). This may be due to cultural or self-induced restrictions on daily activity levels in girls (49,50). A falling VO_2 max is also observed in studies on pre-adolescent and adolescent girls from different countries (51-53).

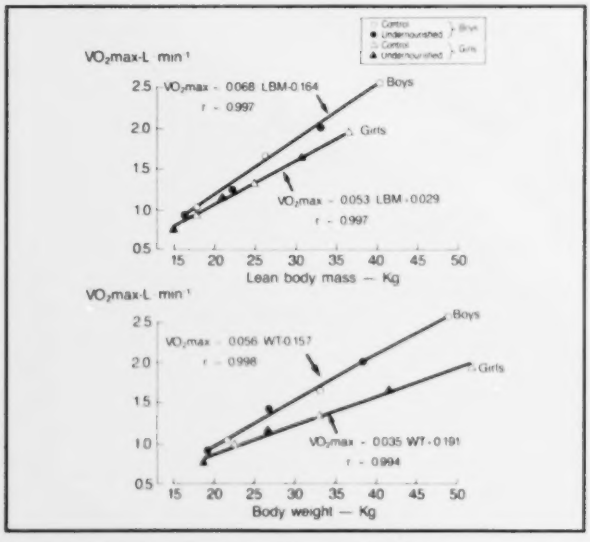

Figure 10. Average values of VO_2 max (L/min) as a function of body weight and lean body mass in nutritionally normal and marginally malnourished Colombian boys and girls 6-16 yrs of age (From Spurr and Reina (49)).

The effect of chronic marginal malnutrition on growth and VO_2 max in boys and comparison with adult males in Colombia and the U.S. are summarized in Figure 11 which

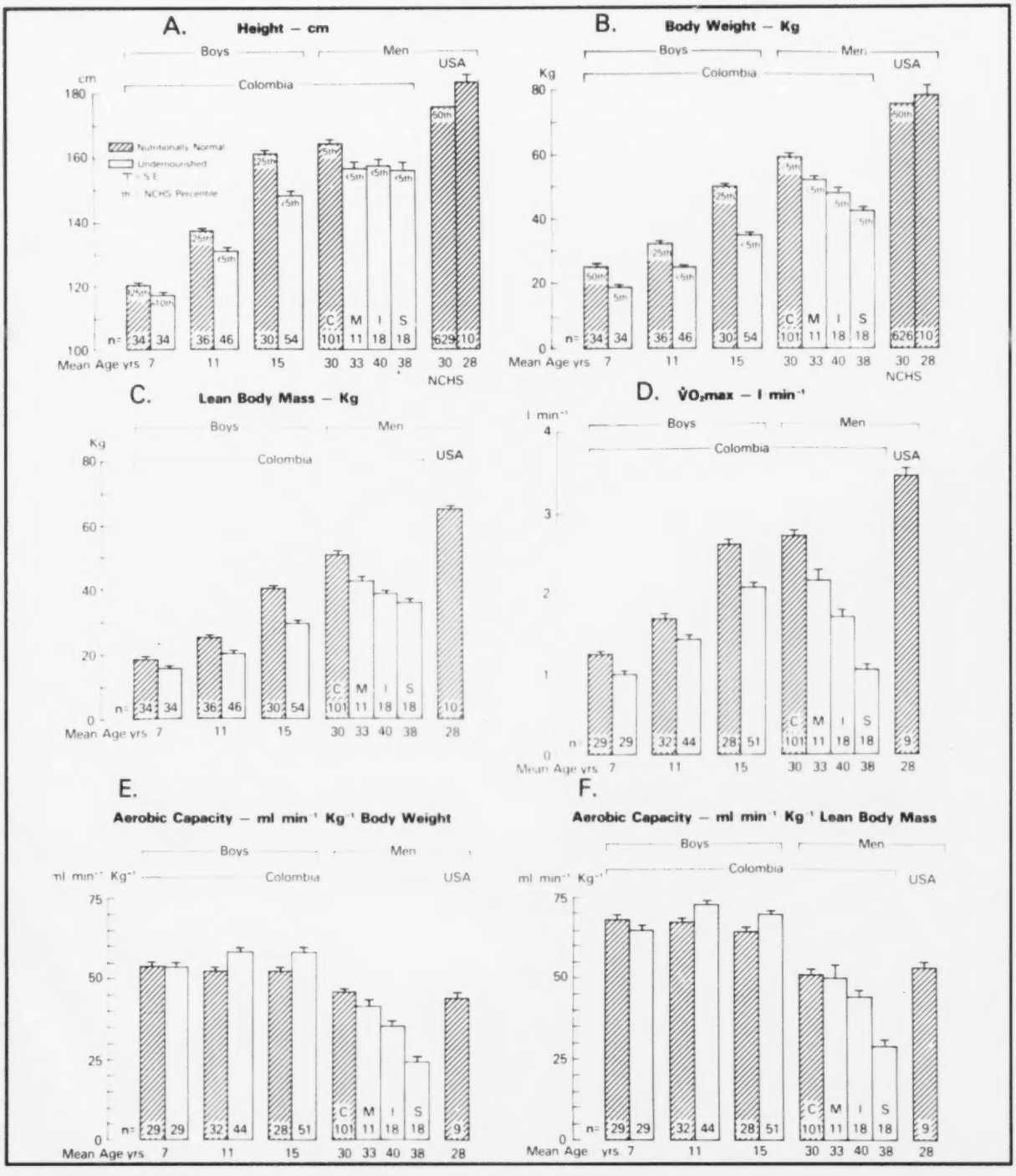

Figure 11—Body composition and maximum work capacity of normal and undernourished Colombian school-aged boys and adult agricultural workers classified as nutritionally normal control subjects (C) or as moderate (M), intermediate (I) and severe (S) in the degree of their nutritional deprivation. Also shown are the values for a group of North American men and the U.S. NCHS 50th percentile values of weight and height for adult men (From Spurr (54)).

shows height, weight, LBM and three expressions of VO_2 max in our various Colombian subjects and 10 North American men (54). The development of body size and of LBM was significantly attenuated during growth as is the VO_2 max (L/min) which is <85% than that of the normal subjects (Fig 11D). When expressed as ml/min/kg body weight the two oldest groups of undernourished boys have higher aerobic capacities than the normal boys (Fig 11E), which is due to the fact that they are in better physical condition than their nutritionally normal counterparts since the same thing occurs when the data are expressed in terms of ml/min/kg LBM (Fig. 11F). The possible reasons for this are speculative and not germane to the present topic. They have been discussed elsewhere (55).

Summary

There is no question that the reduced oxygen carrying capacity of arterial blood which occurs in acute or chronic anemia results in a reduction in the physical work capacity of individuals, with obvious implications for productivity in heavy physical work. Despite this restriction, sub-maximal work can sometimes be performed to surprising intensities. There is some evidence that in children there may be a greater adaptation to low hemoglobin than occurs in adults.

Iron deficiency in the experimental rat affects muscle by reducing the activities of oxidative and glycolytic enzymes and myoglobin. When anemia associated with iron deficiency is corrected, the reduced VO_2 max is also corrected but deficiencies in endurance capacity remain. In humans the separation of physical work capacity (VO_2 max) and endurance is not so clearly demonstrated. The muscle enzyme changes found in rats have not been shown in acute (about 4 weeks) or chronic iron deficiency of humans. Correction of the anemia results in correction of both VO_2 max and endurance, indicating a possible species difference between humans and rats.

In both acute and chronic undernutrition, the reduced VO_2 max which results is proportional to the degree of malnutrition and is primarily related to the body size of the individual as reflected in the lean body mass or muscle mass. The anemia usually associated with malnutrition may account for only about 20% of the reduced physical work capacity.

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IRON IN THE CENTRAL NERVOUS SYSTEM

Theodore L. Sourkes
Departments of Psychiatry and Biochemistry,
Faculty of Medicine,
McGill University,
Montreal, Canada

Nature and distribution of iron compounds in the nervous system

Iron is present in nervous system tissue in two forms: as compounds bearing the heme prosthetic group, and as compounds with iron in some other linkage than to the heme molecule. Analysis of the brain by region demonstrates that the basal ganglia and some associated structures, constituting the extrapyramidal motor system, are particularly rich in iron. This is illustrated by the data of Table 1 for human brain. Most of the cerebral iron is in the non-heme form, and a small portion of it is present in certain enzymes (8). The two valence states of ionic iron function in the oxidative actions of these enzymes, many of which are concerned with terminal metabolism of carbohydrates and related substances. For example, succinate dehydrogenase and aconitase (aconitate dehydratase), both catalysts in the tricarboxylic acid cycle, are iron enzymes. Other iron-containing dehydrogenases have glycerol-3-phosphate and NADH as substrates. Cytochrome oxidase and catalase are iron-heme enzymes. Another group of iron-containing enzymes act in transformations of certain amino acids and amines. Among these are hydroxylases acting upon tyrosine and tryptophan, indoleamine-2,3- dioxygenase, aldehyde oxidase and hypotaurine dehydrogenase. One must recognize here a role for iron in selected aspects of neurotransmitter synthesis and metabolism.

The data in Table 1 provide information about the distribution of three important iron-containing enzymes. Succinate dehydrogenase is clearly rich in regions of high cellularity, i.e. where oxidative metabolism is highest, and low in white matter. The two aromatic amino acid hydroxylases have different distributions from one another and from succinate dehydrogenase. For example, tyrosine hydroxylase is present in largest amount in those parts of the brain where dopamine is a principal neurotransmitter, and must be provided in adequate amounts for regulation of tone and posture of the limbs, as well as other functions. Tryptophan hydroxylase, on the other hand, is found in the serotonin-synthesizing areas of the brain, i.e. the midbrain raphe nuclei and their terminal projections. The terminals can be isolated as synaptosomes, and these structures in adult rats have been found to contain about 60-90 microMolar Fe; there is only about one-third of this amount in weanling pups (9). Presumably some of this synaptosomal iron is associated with aromatic amino acid hydroxylases.

Non-heme iron

The non-heme iron compounds of brain and other organs can be classified according to their ease of extractability from tissue (Table 2), as well as by their properties, where specific entities have actually been isolated for structural studies. Thus, some iron is readily reactive, and corresponds to the fraction that is (a) loosely bound to low-molecular-weight compounds (10) and (b) detectable by use of chelating agents or by the 'bleomycin' assay (11,12). By means of this sensitive assay, it has been shown that

the concentration of catalytic iron in the tissues available for "Fenton chemistry" is of the order of 5 microMolar. Transferrin gives up its iron readily. Its two atoms of Fe(III) per mole of protein are released in the acidic milieu of the lysosomes (13). As for specific compounds making up this heterogeneous group, the simplest is ferrous iron, which may behave as a free radical (14), generating the very reactive hydroxyl radical in the reaction:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + {}^{\cdot}OH$$

Ferritin is a protein capable of binding variable amounts of ferric iron, indeed, as much as 4500 atoms per mole. This iron is released, through the action of superoxide anion or ascorbic acid, in the reduced form of Fe(II). Hemosiderin, a protein containing iron and lipid, may be a degradation product of ferritin. Its iron appears to be fairly tightly bound. Iron-sulfur proteins function in electron transfer. One such protein is adrenodoxin, which acts in concert with cytochrome P450 in certain steroid transformations in the adrenal gland (15). Molecules of this kind are presumably also involved in the brain, in steroid transformations in that organ (16-18). These syntheses produce neurophysiologically active steroids (16,19-21). Lipofuscin, the so-called 'age-pigment', is actually a secondary organelle that contains relatively high concentrations of metals, including iron and copper (22). Finally, there are the iron-containing enzymes mentioned above. The form of attachment of iron and its catalytic role are still being explored in these. Melanin is a type of polymeric ion-exchanger, capable of binding metal ions. Out of ten such ions tested for incorporation into melanin from solution, iron was taken up to the greatest extent (23). This property is the core of a novel hypothesis about the pathogenesis of Parkinson's disease (24).

Table 1. Distribution of non-heme iron in mammalian brain

Region	Total iron	Non-heme iron		Succinate dehydrogenase	Tyrosine hydroxylase	Tryptophan hydroxylase
	man[a]	man[b]	man[c]	dog[d]	rhesus[f]	cat[g]
Cerebral cortex	29-77	63	31-50[e]	44-93[e]	1	1
White matter	19-37	42	42	3-14	3	
Globus pallidus	16-266	146	213		36	
Caudate nucleus	54-217	93	93	137	109	23
Putamen	15-213	122	133		112	
Substantia nigra		130	184		141	
Locus coeruleus		54				
Thalamus	32-71	49	48	90	4	9
Hypothalamus					8	14
Hippocampus	24					
Cerebellum	20			100	0	0.7
Medulla oblongata			14			
Spinal cord		10				

[a]Micrograms/g, (1,2)
[b]Micrograms/g (3)
[c]Micrograms/g (4)
[d]Range of means for different areas of cerebral cortex
[e]Relative values (5)
[f]n-mole of product formed/g per hour (6)
[g]n-mole of product formed/g per hour (7)

Table 2. Iron compounds in nervous tissue

Heme iron

- Various cytochromes
- Cytochrome oxidase
- Catalase

Non-heme iron

- Characterized by extractability
  - Readily reactive (e.g. with potassium ferrocyanide in acid; or with ammonium sulfite). Can be detected by use of chelating agents. Iron loosely bound to low-molecular-weight compounds. The fraction of tissue iron that is extracted with acetate buffer, pH 4.5 represents about one-third of the non-heme iron in brain

Soluble in strong acid (e.g. extraction with 5N HCl heat). This fraction contains the total non-heme iron, with virtually no heme iron.

Species

- Ferrous iron
- Ferritin
- Hemosiderin
- Transferrin
- Ferredoxins
- Lipofuscin
- Succinate dehydrogenase
- Tyrosine hydroxylase
- Tryptophan hydroxylase
- Aconitate dehydratase
- Deoxyribonucleotide synthetase

Heme iron

Heme synthesis takes place in the mitochondria. Some of the product remains in those organelles, some is exported. Among the iron-heme compounds are the cytochromes and cytochrome oxidase (Table 3). Cytochromes a, b and c, and the oxidase are involved in electron transport. As in other tissues, brain microsomes contain small amounts of cytochrome b5 and P450 (27,30). These serve in mixed-function oxidases, acting in the metabolism of steroids and foreign compounds such as drugs (xenobiotics). Their action renders these substances more polar, through oxidation and excision of side chains (dealkylation). Cytochrome b5 is linked to the oxidation of NADH by the flavoprotein NADH-cytochrome b5 reductase. Cytochromes b5 and P450 both increase with age, but the amounts, particularly of the former, vary from species to species. For example, rat brain contains much more than rabbit brain. Beef and guinea pig brains are relatively low in cytochrome b5. In any case, the amounts in brain are much lower than in liver. Thus, beef liver contains 1350 pmol/mg protein of cytochrome b5; the brain has only 12 pmol/mg protein. This is also true of the microsomal content of cytochrome P450. Cytochrome c is absent from microsomes.

Histochemical distribution of iron

The distributions of iron, as set out in Table 1, are based upon relatively gross dissection into the designated regions, and therefore provide limited information. For sharp focussing, histochemical detection is necessary, and this has now been combined by Hill (31,32) with computer- assisted densitometry. Hill has characterized the iron content of over 70 specific anatomical areas of the rat brain. For example, the greatest concentrations of the metal occur in certain limbic structures (the islands of Calleja and the lateral superficial border of the olfactory tubercle) and in organs surrounding the ventricular cavities (organum vasculosum of the lamina terminalis, median eminence, hypothalamus, area postrema, and others). These circumventricular organs possess a modified blood-brain barrier. Somewhat lower but still relatively high concentrations occur in some structures associated with the basal ganglia (globus pallidus, entopeduncular nucleus, pars reticulata of the substantia nigra) and in certain hypothalamic nuclei (supraoptic and paraventricular nuclei) and in the suprachiasmatic nucleus. The latter structure is involved in circadian rhythms, and it is of interest that the pineal gland is also rich in iron. The striatum, red nucleus and raphe nuclei are in a category with less iron. The hippocampus is also in this group, according to Hill and Switzer (31), although analysis of the dissected hippocampus is reported to be relatively rich in iron (33). The histochemical method of Hill detects iron in both neuronal cell bodies and glia, especially the oligodendrocytes; some is found in myelin membranes also and, as already mentioned, in mitochondria and microsomes, the latter representing largely the endoplasmic reticulum.

Uptake of iron into the brain, and transport

Both neurons and glia in culture (i.e. in the absence of the blood-brain barrier), incubated in the presence of ferritin partially saturated with iron, take up the metal in a steady fashion with time (34). In intact brain the mechanism for importing iron seems to involve specific transferrin receptors on the luminal surface of the capillary endothelium (35,36). Subsequent movement of the transferrin molecule or the transferrin-receptor complex into brain cells results in the release of the iron. The overall processes of delivery of iron into the brain and its loss from that organ, i.e. the turnover, are extremely slow (37), a condition that makes it difficult to alter the iron status of the brain. In fibroblasts, release takes place in the acidic environment of the lysosomal cytoplasm (13). This probably pertains also to cerebral cells, inasmuch as their uptake of iron in culture is inhibited by chloroquine (38), a drug that blocks entry of certain substances into the lysosomes and also raises the intralysosomal

Table 3. Concentration of cytochromes in adult brain

Brain organelle	Constituent	Cytochrome content (pmol/mg protein)	
		Rat	Beef
Mitochondria[a]	Cytochrome a	204	258
	a_3	248	
	b	121	230
	c	287	142
	c_1		134
Microsomes	Cytochrome P450	35[b,c,d]	
	b_5	87[b]	12[e]

[a]Data for rat mitochondria: reference 25; for beef mitochrondria: reference 26.

[b]Reference 27. Content of cerebrum is shown. The cytochrome b_5 content of the cerebellum is about 30% greater than this.

[c]Warner et al. (28) give the concentration of P450 in whole brain of rat as 90 pmol/g of tissue. For cerebral cortex the value is of the order of 60 pmol/g. The cerebellum is unusually high, with a mean of 366 pmol/g.

[d]Percy and Shanley (29) give the P450 content as 25 ± 6.3 pmol/mg protein (mean ± SD, n = 6) for mitochondria, and 12 ±6.2 pmol/mg protein for microsomes. Corresponding figures for liver are 130 and 430 pmol/mg protein, respectively.

[e]Reference 30. This value is for the cerebral cortex of rabbit. Cytochrome P450 was not detectable.

pH (13). Transferrin receptors have an interesting distribution, in that they occur in the neutrophil of many areas poor in iron (e.g. caudate-putamen, pons), but containing neurons that project to iron-rich sites. This implies that the iron is taken up, perhaps through the intermediation of glial cells, into the dendrites of these neurons and is then transported in some form, possibly in combination with transferrin, down the length of the axon. In the terminals of serotonergic neurons, serotonin binds with high affinity to a soluble protein, and this binding is greatly enhanced in the presence of up to 10^{-4} Molar iron. Greater concentrations inhibit the reaction. The serotonin-binding protein apparently serves in storage of that amine in central and peripheral neurons, with ferrous iron attached to the protein (39,40). In fact, analysis of synaptosomes prepared from rat brain indicates that the neurotransmitter-storing vesicles are enriched in iron compared to the extravesicular fluid of the synaptosomes (41). Normally, iron is available in these locations, for Kaladhar and Narasinga Rao (9) have measured 0.2 micrograms of iron per gram of original tissue in isolated synaptic vesicles of rat brain. Newborn pups had only one-third as much. Iron released from the terminations would then become available to the oligodendrocytes (36). These cells contain transferrin (42,43), some of which may originate in the circulation, and they also have a mechanism for its synthesis, viz. transferrin-mRNA (44). Transcription of the transferrin gene takes place in rat fetal organs except brain, but the process commences in that organ just after parturition and increases in pace during early growth (45). In a mutant rat with histopatho-

Table 4. Changes in brain proteins resulting from dietary iron deficiency

Animals on experimental diet at	Fe content of D/C diets (mg/kg)[a]	Protein, or enzyme activity	Duration of deficiency (weeks)		Reference
			4/5	8/9	
80 g body	not stated/250	Monoamine oxidase		82	(54) weight
Aldehyde	dehydrogenase	nc			
		Tryptophan hydroxylase	nc	84	
3 weeks of age	50/250	Dopamine D_2-receptor (CN[b])	46		(49)
		Albumin (CN)	214		
		Albumin (NA)	146		
		Neuron-specific enolase (NA)	122		
		Glial fibrillary acidic protein (CN)	62		
3 weeks of age	35/390	Total protein	nc[c]		(55)
		Glutamate decarboxylase	nc	86[d]	
		GABA-transaminase	76	72	
		Glutamate dehydrogenase	86	68	
		Isocitrate dehydrogenase:			
		NAD-linked	91	76	
		NADP-linked	nc	nc	
		Succinate dehydrogenase	nc	nc	

[a]D=deficient diet; C=control diet

[b]CN=caudate nucleus; NA=nucleus accumbens

[c]No significant change

[d]Statistically significant change detected. Percent of control value shown.

logical changes in the oligodendrocytes and, consequently, deficient myelination, the brain is essentially devoid of transferrin, even though hepatic synthesis of the protein is normal (46). Transferrin has growth-promoting activity for certain mammalian cells, and may be identical to the neurotrophic factor that is transported from nerve to muscle. In fact, this musculotrophic substance requires iron for its activity (47), so that transferrin synthesized in brain may serve in the neural regulation of muscle tissue and its activity (44,45).

Neurochemical changes in iron deficiency

Dietary deficiency of iron in rats results in two important types of change in the chemistry of the brain, namely, a decrease in its iron content, and specific decreases in some of its proteins. In the course of the deficiency the non-heme iron content of the brain declines progressively, and reaches about half the normal value 5 weeks after weaning rats to a diet containing only 50 mg of iron per kilogram (48,49). Among the sites undergoing this decrease are the terminations of the neurons. The synaptic vesicles separated from these structures in the brain of pups born to iron-deficient dams have less than 40% of the control complement of the metal (9). This is associated with a decreased rate of uptake of serotonin by the vesicles (50). It is not known if this results in a reduction in the amount of serotonin complexed with the ferroprotein that binds it in vesicles (v. supra).

In regard to brain proteins, it has been recognized for some years that lack of iron results in defective protein synthesis in several organs (51). Mitochondrial proteins, among others, are involved (52,53). Certain brain proteins are also affected by the deficiency (51), notably dopamine D_2- receptors, detected by a decrease of spiperone-binding in the caudate nucleus of the rat brain (48,49), and glial fibrillary acidic protein, separated by gel electrophoresis (49). These are listed with some other proteins in Table 4. The decrease of the fibrillary protein, a product of astrocytes, may signify a relative loss of glial cells, but histological evidence of this would be needed. The reduction in titer of dopamine receptors suggests important functional consequences and, in fact, motor activity in response to the potent dopamine agonist apomorphine is very much reduced (49). Monoamine oxidase, tryptophan hydroxylase, and the NAD-linked isocitrate dehydrogenase undergo small decreases in the brain, but only after prolonged deficiency (Table 4). Gamma-aminobutyrate transaminase (GABA-T) activity decreases significantly by 4 weeks of deficiency and remains at that level for at least another month. Aldehyde dehydrogenase and succinate dehydrogenase are both reported to be resistant to prolonged dietary iron deficiency.

Some brain proteins are increased as a result of the lack of iron. These include albumin in both caudate nucleus and the nucleus accumbens, and the neuron- specific enolase in the latter structure (49). It is possible that the enolase, a marker of the anerobic phase of glucose metabolism leading to the synthesis of ATP, may increase in response to the poor delivery of oxygen to the brain resulting from anemia.

Iron deficiency also causes variable changes in the blood-brain barrier. Thus, valine transport declines, but insulin entry into the brain is facilitated (56).

Some of the cerebral changes resulting from iron deficiency are reversible, others are not. The apparent irreversibility is particularly evident the younger the animal at the time the deficient diet is introduced. Thus, rats given the iron-deficient diet at 3 weeks of age are later able to recover from the deficiency when supplemented with the metal. Younger rats are unable to do so (57).

Monoamine metabolism

Like tryptophan hydroxylase (Table 4), cerebral tyrosine hydroxylase does not decrease during experimental iron deficiency (58). However, this enzyme increases in amount in the adrenal gland of the deficient rat, for which the stress caused by anemia is probably responsible (59). This could ex-

plain the elevated urinary excretion of norepinephrine in the urine of iron-deficient children (60) and rats (61). While increased synthesis of norepinephrine could contribute to its increased urinary output, decreased metabolism may also be important. This possibility stems from two of the studies that introduced the question of the role of iron deficiency into neurotransmitter physiology, and that demonstrated how quickly catabolism of monoamines responds to the withdrawal of iron from the diet (62,63). In humans the key enzyme in metabolism of catecholamines and serotonin, monoamine oxidase, can be readily measured in the blood platelets. It has been shown that in simple iron-deficiency anemia in man the platelet enzyme declines in activity, and can be restored to normal levels with adequate iron therapy (64). The regulation of the serotonin content of the brain is undoubtedly multifactorial, but in relation to iron deficiency, two processes can be highlighted, namely a decrease of monoamine oxidase activity, which would be protective and could lead to higher levels of the amine in the brain and binding by the serotonin-binding protein, whereby loss of iron could result in less capacity for storing the amine and, consequently, maintaining smaller amounts of serotonin in the brain tissue. Thus far, Youdim and Green (65) have reported a decrease of serotonin in the brain of iron-deficient rats, whereas Mackler and Finch (66) assert the opposite.

GABA metabolism.

The metabolism of glutamate and GABA is affected to some extent by simple nutritional iron deficiency in rats. Glutamate dehydrogenase is decreased significantly by the fourth week of iron deprivation. A small decrease of glutamate is noted at six weeks (55). According to recent evidence these changes may not be as relevant to the metabolism of GABA as previously thought, inasmuch as that amino acid is apparently derived, not from the major pool of glutamate in the neuron, but from a minor compartment which could behave quite differently physiologically (67). GABA is formed from glutamate through the action of a specific decarboxylase, and is metabolised by transamination (GABA-transaminase) to succinic semi-aldehyde, a precursor of succinic acid. Glutamate decarboxylase and the transaminase both undergo decreases in the course of iron deficiency, although in the case of the first of these enzymes the loss of activity is minimal even after as long as 8 weeks (55; Table 4). These enzymes are not linked to iron.

Despite this tenuous relationship, Hill's histochemical studies show "that there is a considerable overlap in iron-rich brain areas and those regions in which GABA neurons terminate" (31). The relationship is pointed up by the effect of increasing the GABA content of the brain through inhibition of GABA-transaminase, achieved by injecting gamma-vinyl-GABA into specific regions of the rat brain. In one experiment Hill injected the inhibitor into the globus pallidus and the adjacent region of the striatum. This caused a loss of iron in the ipsilateral pallidum and the substantia nigra pars reticulata (68). There was no effect upon the iron content of the nigra following an electrolytic lesion of the globus pallidus, a measure that would of course eliminate the cell bodies of many of the GABAergic neurons. The mechanism of the nigral iron loss is not clear, but presumably it involves the increased neuronal content of GABA in the striatum and pallidum, both of which project GABAergic fibers to the substantia nigra pars reticulata in the rat (69). This might not pertain in primates, where pallido-reticulata fibers are exceedingly sparse (69,70).

Neuropathology of iron

Hemochromatosis.

This condition of excessive iron accumulation in the liver and other organs sometimes manifests itself through predominant neuropsychiatric symptoms (71). Neurological changes are represented in the main by progressive bilateral deafness (72). The relationship of iron overload to these symptoms is

unclear. The pigment that accumulates in the tissue is hemosiderin; its high iron content suggests that it may be a derivative of ferritin. A model of hemosiderosis (iron accumulation) can be prepared in animals given large doses of an iron-polymaltose complex (73). In order for the metal to penetrate into the brain, the animals are first treated with the EAE (experimental allergic encephalomyelitis) antigen to render the blood-brain barrier more permeable.

Parkinson's disease.

A suggestion made many years ago by Earle (74) that the cerebral iron content is elevated in Parkinson's disease has been confirmed in recent years by detailed studies of brains obtained at post-mortem. The evidence for a specific increase of iron, particularly Fe(III), in the substantia nigra (but not other regions of the brain) is quite clear (75,76). This increase is found in brains severely affected by the disease, but not in the milder cases (75). The evidence for a change in the iron content of the globus pallidus, ordinarily rich in the metal, is as yet controversial. Jenner and his colleagues (cited in reference 77) have reported a general decrease in the ferritin content of the Parkinsonian brain, but according to Riederer and his colleagues (75) the only change in this substance is observed as an increase in the substantia nigra.

The iron-linked enzyme tyrosine hydroxylase, ordinarily high in concentration in the striatum, decreases in Parkinson's disease (78-80). Biopterin has been tested in patients with the disease, but without any therapeutic success. On the other hand, intravenous administration of an iron preparation is said to provoke a favorable response (81).

As already mentioned, the change in the nigral content of iron figures in current hypotheses about the pathogenesis of Parkinson's disease (See Youdim, This Symposium).

Restless legs syndrome.

A not infrequent side effect of treatment with neuroleptic drugs is akathisia, characterized by "an inner restlessness of mind and body" (82). The motor disquiet is particularly apparent in the constant movement of the legs. The response to antiparkinsonism medication is poor. This extrapyramidal syndrome is observed also in subjects who have not received neuroleptics, but in these cases the psychic component is absent. Ekbom (83) tried to trace the pathogenesis of the syndrome, and concluded that it stems from various causes. In his series the incidence of iron-deficiency anemia was high (24%), and these cases responded to treatment with injectable iron. More recently, an examination of serum iron levels in patients receiving antipsychotic medication demonstrated an inverse relationship between akathisia rating and serum iron concentration (84). As a group, the akathisic patients had a significantly greater iron-binding capacity, but a lower degree of saturation than in patients without akathisia. It is possible that the lowered stores of iron could result in diminished numbers of dopamine D_2- receptors, as found in rats functionally deprived of iron (48,57) so that transmission along dopaminergic pathways is less efficient.

Hallervorden-Spatz disease.

This is a recessive, inherited disorder of unknown pathogenesis (85). The globus pallidus suffers some atrophy, with accumulation of pigment. Necropsy analysis reveals large increases in the tissue content of iron, but copper, zinc, manganese and calcium are also increased. In contrast to this, the iron content of the caudate nucleus, putamen, substantia nigra and cortex is very low (85). Park and colleagues (86) observed an increase in nigral as well as pallidal iron, and postulated that the pigment represented a non-enzymic conversion of lipofuscin, catalyzed by iron and copper in peroxidative reactions, to "neuromelanin".

Problems of CNS iron research.

Our knowledge of iron metabolism and function in the CNS is gradually improving, but there are many places where neurochem-

ical investigation is needed as an aid to the solution of practical problems. The following points come to mind:

1. The mechanism of transfer of iron from blood across the endothelium of brain capillaries, and thence into neuronal and glial cells. This may provide clues as to how to accelerate uptake when there is evidence of cerebral dysfunction in iron deficiency. Lipophilic molecules incorporating iron would be a possibility to investigate.

2. The nature of the glutamate compartment giving rise to GABA. This would entail, in the present context, determining whether any effect of iron deficiency stems from a change in some remote precursor or enzyme in a tightly regulated metabolic corridor starting with carboxylation of pyruvate and proceeding along the following sequence: oxaloacetate, citrate, cis-aconitate, isocitrate, alpha-ketoglutarate, glutamate. The isomerisation of citrate is catalyzed by the iron(Fe^{2+}) enzyme aconitate hydratase. It is conceivable that some peculiarity of compartmentation within the neuron renders this minor complement of the enzyme more readily susceptible to iron deficiency than the major portion, which serves in the catabolic compartment of the tricarboxylic acid cycle.

3. The relation of GABAergic neurons to iron content of the brain. This would require tests of GABA agonists and antagonists in animals with evaluation of the regional iron content of the brain. Differences in GABAergic pathways between species, e.g. the connections of the basal ganglia and associated structures (69), require that such studies not be restricted to small animals, but should be extended to primates as well, in order to assess better the relevance of this GABAergic function to cerebral iron metabolism in man.

4. Finally, information on the distribution of iron in the human brain in health and in disease states is inadequate, especially in relation to neuroanatomical pathways. Programs making use of necropsy tissue from brain banks would undoubtedly provide novel and useful information in this area.

The method of neutron activation for analysis of iron (cf. reference 2) is convenient, and the radioisotope of iron formed (^{59}Fe) has a half-life of 44 days.

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RELATIONSHIP OF BRAIN IRON TO DOPAMINERGIC NEUROTRANSMISSION AND TO THE GENESIS OF PARKINSON'S DISEASE

Moussa B.H. Youdim

Uniformed Services University of Health Sciences
School of Medicine
Department of Pharmacology
and
National Institute of Health
Laboratory of Cell Biology and Genetics
NIDDK
Bethesda, MD 20205

The brain, like the liver, contains a substantially greater concentration of iron than of any other metal (1). The notion that brain iron content could not be altered may have been one reason why the roles of this metal in brain function and dysfunction were not considered until relatively recently. This is rather surprising since iron is an essential participant in many metabolic processes including (a) DNA, RNA and protein syntheses; (b) as a cofactor of many haem and nonhaem enzymes; (c) formation of myelin and (d) the development of neuronal dendritic tree. A deficiency of iron metabolism would therefore be expected to alter some or all of these processes (2,3). But by contrast, an excessive accumulation of tissue iron may lead to oxidative stress via the formation of oxygen free radicals which can be highly cytotoxic (4,5).

We must, however, now give careful attention to the role of iron in brain physiology, since tissue iron-deficiency and iron-overload represent the most prevalent metabolic disorders in the world. The essential participation of iron in brain development and metabolism (deficiency or excess) could have profound, even long term irreversible consequences (6,7,8). In no way is this paper intended to be a review of the subject but special consideration will be paid to the recent developments. The reader's attention is therefore directed to the previous, more recently published studies (9).

Iron Deficiency (ID)

The behavioral consequences of ID in adults and children include apathy, lethargy, irritability, listlessness, fatigue, lack of concentration, inattention, pagophagia, hypoactivity, altered sleep pattern, pica, and decreased IQ, cognitive and attentional processes (10). Until recently most of the reports related to behavioral alterations were treated with a great deal of skepticism, even though iron therapy could reverse some of the symptoms. In the past few years, however, both animal and human studies have been initiated to obtain more direct evidence for lowered brain iron and central nervous system induced behavioral abnormalities (1,11).

The summary and careful conclusions of human and animal studies at the recent conference on iron deficiency and behavioral development have resulted in the participants endorsing the following statements (12).

"Many studies have shown an association between iron deficiency anemia (IDA) and less than optimal behavior in infants and children as demonstrated by lower scores on tests of development, learning, and school achievement. A problem with the interpretation of these studies is that IDA is associated

with other adverse environmental and nutritional conditions which may independently affect behavior. However, new studies using randomized designs with appropriate controls, many of which were presented in this conference, have shown that iron therapy in preschool and school-aged children with IDA results in improvement in certain behavioral tests. This research on children and a number of animal studies suggest that IDA is causally associated with less than optimal behavior. Therefore, it is important that IDA be prevented and treated in all children. Because the specific mechanism and functional significance of these behavioral changes are not completely understood, further studies are essential both to clarify the effects of IDA itself and to determine the importance of lesser degrees of ID in children (12)."

The implications of these findings for future clinical treatment, public health innovations and basic research are most obvious, considering the enormously large number of subjects affected.

The most important and telling aspects to be considered in iron-deficiency are the critical and vulnerable periods of brain development in the infant, when the deficiency occurs and which may lead to some biochemical derangement? The result of which could be the occurrence of abnormal behaviors or the absence of normal responses?

Animal models have always been useful in determining brain function in response to nutritional deficiency or excess of iron. Although such models inevitably have their own limitations and faults, nevertheless they have been extremely useful in understanding some of the biochemical consequences (13). The studies on nutritional iron-deficiency induced in rats have not escaped close scrutiny (13). They have added a wealth of new data to the understanding of the functional role of iron in brain neurotransmission. To a large extent, this has been attributed to the unique distribution of iron in the subcellular fractions and different brain regions. The highest brain iron concentrations are found in the globus pallidus, substantia nigra, putamen, dentate gyrus, interpeduncular nuclei, caudate nucleus, red nucleus, and ventral pallidum. This is true for both human and animal brains (1,14). These regions constitute the brain areas with highest concentrations of neurotransmitter substances such as GABA (gamma-amino butyric acid, dopamine and neuropeptides enkephalins, endorphins, somatostatin, etc.).

It is now apparent that indeed nutritional iron-deficiency in rats does induce a reduction of brain iron (15). However, the extent of iron concentration diminution is dependent on the age at which the animals are made iron-deficient and the brain regions examined (16). Thus adult (48 day old) rats are much less vulnerable to reduction of brain iron concentration than new born (10 day old) animals. In contrast, rehabilitation of brain iron by iron supplementation is extensively more difficult in new born than in the adult rat. Even six months of iron therapy does not restore the brain concentration in iron deficient new born (10 day old) rats (6,17,18).

The extensive biochemical and behavioral studies of iron-deficient rats of different ages have demonstrated down regulation (subsensitivity) of dopamine neurotransmission in correlation with reduction of brain iron concentration (19). The significance of this association has been highlighted by our recent studies using a new iron-deficient diet, where haemoglobin and serum iron in rats can be significantly reduced without an alteration in tissue (liver and brain) non-haem iron. In these animals, dopamine neurotransmission as measured by (a) behavioral response to dopamine agonist, apomorphine, (b) dopamine D_2 receptor number in caudate nucleus and (c) dopamine turnover, is unaltered (20). In contrast, the diet which induces true tissue (brain and liver) iron-deficiency is associated with a reduction in postsynaptic dopamine D_2 receptor number (Bmax) and the behavioral response to apo-

morphine (6). The inability to restore brain dopaminergic neurotransmission and dopamine D_2 receptor number in iron-deficient new born (10 day old) rats has led us to implicate the greater specific vulnerability of dopamine neurons in this period. Although the biochemical events leading to reduction in dopamine D_2 receptor in brain ID are not fully understood, they are thought to be related to an alteration in the rapid postnatal development of dendritic tree, which takes place in the first 25-35 days of a rat's life. It is in this period that the rapid increase of myelinization, lipid deposition, brain iron deposition and dopamine neuron proliferation occur. This is demonstrated by the increase of dopamine D_2 receptor, dopamine synthesis and dopamine metabolizing enzymes. Preliminary studies with brains examined at this period indicate interference in dopamine D_2 receptor formation in iron-deficiency (6). The direct role of iron in this process cannot be a certainty but intraventricular injection of $FeCl_3$ induces both biochemical and behavioral supersensitivity of dopamine D_2 receptor (21). However, for the physiological dysfunction of brain, leading to abnormal behavior responses in iron-deficiency, this is not important. What matters is the deficiency of essential metal and the consequences of that deficiency.

Dobbing (22), in comparing rat and human brain development (the phase of rapid proliferation, growth and myelinization) has suggested that 25 day postnatal rat is equivalent to the first 2 to 3 years in human life. It is in this period of human life where the major portion of iron-deficiency is noted. Thus the implications from animal studies for human brain development and behavioral function are obvious. These may, therefore, be the most critical and vulnerable periods of brain growth when the essential role of iron comes into play.

If the animal model of nutritional iron-deficiency, with its biochemical lesion of dopaminergic neurotransmission, is to be linked to human brain in this condition, there is a clear need to know which developmental phase is being examined. This is crucial to the understanding of the possible biochemical mechanisms.

Iron-deficiency and learning

The major symptoms of iron-deficiency as related to brain function in humans (infants and adolescents) are the persistent low mental and motor test scores in infants, even after iron treatment, and cognitive changes in school children (23-28) that seem to improve with iron treatment. At present it is less clear whether the behavioral alterations associated with iron deficiency in the infant-toddler age groups are cognitive or correctable with treatment. Thus, it has been challenging to set up an experimental model of animal iron-deficiency to examine any modification in cognitive function such as learning extinction and exploration. The effect of iron on learning capacity (active and passive avoidance) of iron deficient rats has been studied before in off-spring of iron-deficient mothers and in very young (10 day old) rats exposed to an iron deficient diet (17,18). However, these studies have been criticized for the use of shock avoidance test which does not measure true learning capacity. Nevertheless, these animals are less responsive (open field) and display decreased ambulation and longer re-entry latencies on shock avoidance. Subsequent to iron-rehabilitation these animals make more intertrial response on active avoidance "learning" (17,18). Improved performance was valid in passive avoidance testing.

We tested the effects of ID on learning capacity using our model, with rats aged 5-6 weeks. Because water and food cannot be used as reinforcers in a nutritional study, the study examined the ability of ID rats' to learn a water maze (29,30). The results showed that ID rats' learning capacity was significantly inferior to that of control rats. The longer that rats were kept on the ID diet, the greater the deficit in learning. One of the most striking findings was that the deficit in

learning was evident even when the level of hemoglobin was not significantly below normal, but serum iron was decreased.

Recently, a similar deficit in learning and memory was found in ID rats trained in a Morris water tank. The task of the rats in this apparatus is to locate a submerged platform (7.5 cm in diameter) in a large water tank (1.0 m in diameter). The tank was filled with water (22 ± 2C) that was made opaque by the addition of powdered milk. Because the animals were unable to see the platform, they had to rely solely on environmental cues in order to reach it. Whereas control rats (n=18) needed 19.6 ± 3.3 trials to reach the learning criterion, ID rats (n=18) needed 26.4 ± 1.1 trials to reach the same level of performance (30)).

At the end of this experiment and at the end of the previous experiment (with the water maze), the rats were fed an iron-supplemented diet and were retested 2, 4, 6, or 8 weeks later. No restoration of learning and/or memory was found. Other aspects of iron deficiency eg, effects on motor activity, thermoregulation, stereotyped behavior, and pain threshold, can be reversed by such treatment (13). However, the deficits in learning and memory remained for > 2 months after iron deficiency treatment. Although the level of hemoglobin in the blood was restored after such a period, the level of brain iron may have remained low for a very long time after iron deficiency treatment (27). This may explain some of the long-term effects of iron deficiency on cognitive function, which cannot be reversed in infants (10,25).

In contrast to our finding of diminished learning capacity in iron-deficiency, Weinberg et al. (17,18) hypothesized an altered threshold of arousal as the etiology for diminished performance when anaemic, and persistence of some alterations after rehabilitation. Passive avoidance test does not necessarily measure learning. Similar fault may be found in the results of the study of Findlay (31) who used 100 day old rats. In the latter case, iron deficient rats as compared to control animals had a shorter escape latency and fewer false entries in passive avoidance shuttlebox task.

These studies, therefore, point to the differences related to the ages of the iron-deficient animals and the lack of consistency with regard to the behavioral measurements made in the animal model of iron deficiency as related to the human situation. The need therefore arises to examine learning, cognition and arousal in animals made iron deficient at ages more appropriate to those consistent with the studies in human infant and adolescent children. Both animal and human studies indicate a critical and vulnerable period in the early development of the brain where iron-deficiency can have irreversible long term effects on the physiology of the brain.

Biochemical mechanism of diminished learning capacity in iron-deficient rats

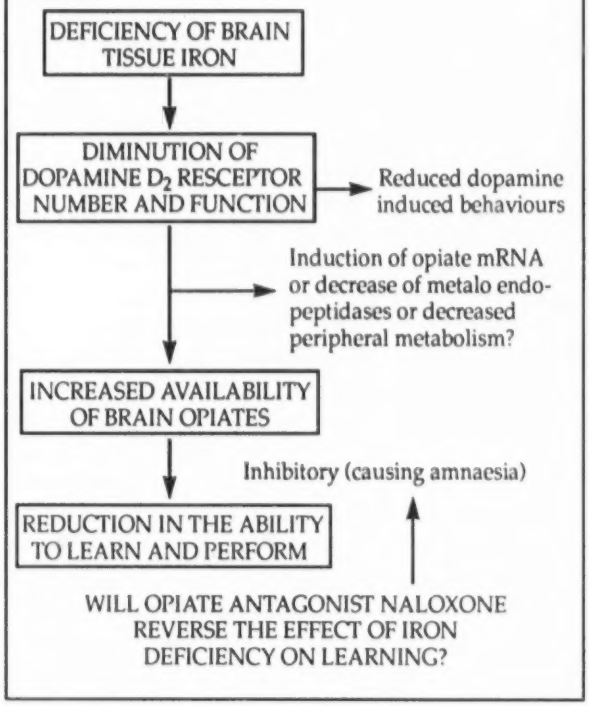
The role of frontal cortex dopamine neurons in cognition and learning process has been well recognized and discussed on previous occasions by numerous investigators. The involvement of dopaminergic neurotransmission in learning cognitive changes in iron-deficient rat has not escaped examination (29). Thus the selective alterations in dopaminergic dependent behaviors have been linked to the learning deficit noted in iron deficient rats (see 10,13,30.

The reduction of dopaminergic neurotransmission in rats, via blockage of dopamine D_2 receptor by neuroleptic (dopamine antagonists) in the brain is associated with increased concentration of brain opiate peptides, enkephalins (31). This has been attributed to increased syntheses of enkephalins resulting from induction of enkephalin mRNA (32). Thus, dopaminergic neurons are thought to be inhibitory to opiate peptide synthesis and function. Indeed recently we have reported increased nonicepton in iron deficient rats, a result thought to be related, which has been attributed to the elevated concentrations of Leu- and Met-

enkephalins and dynorphin B in brain areas, globus pallidus, caudate nucleus, substantia nigra and central grey (31). These are the regions with the highest concentrations of iron. The possibility that elevated opiate peptides may be inhibitory to learning cannot be excluded since these peptides are thought to induce forgetfulness and amnesia. Indeed neuroleptic (dopamine D_2 receptor blockers) do have a negative effect on learning. Naloxone which antagonizes the action of opiate peptides in iron-deficient rats (32,34) may be a useful drug to examine in relation to the diminished learning processes and increased opiates (Fig. 1) in the iron deficient rats.

The behavioral effects of iron-deficiency on learning processes described by us in the animal model of iron-deficiency may be explained by the involvement of iron in the dopaminergic and GABAergic brain regions containing also the highest concentrations of opiate peptides (Fig. 1). Whether or not our hypothesis is correct, the animal model of iron deficiency at different ages does provide us for the first time with a tool to examine the interrelationship of brain iron metabolism with neurotransmitter substances implicated in the physiology of learning processes. It is hoped that what has been learned from the animal model of iron deficiency can be applied to the human condition. Peripheral biochemical markers for brain dysfunctions in iron-deficiency are lacking and are badly needed.

Figure 1. The possible biochemical sequence of events in the brain of iron deficient rats which may be responsible for the reduction in learning processes in these animals.


DEFICIENCY OF BRAIN TISSUE IRON
DIMINUTION OF DOPAMINE D_2 RESCEPTOR NUMBER AND FUNCTION
Reduced dopamine induced behaviours
Induction of opiate mRNA or decrease of metalo endopeptidases or decreased peripheral metabolism?
INCREASED AVAILABILITY OF BRAIN OPIATES
Inhibitory (causing amnaesia)
REDUCTION IN THE ABILITY TO LEARN AND PERFORM
WILL OPIATE ANTAGONIST NALOXONE REVERSE THE EFFECT OF IRON DEFICIENCY ON LEARNING?

Parkinson's disease and brain iron

Despite all that is known about the neuroanatomical and biochemical lesions, namely the degeneration of nigra-striatal dopamine neurons and deficiency of dopamine in Parkinson's disease, the mechanism of its neurotoxicity remains obscure. On numerous occasions the involvement of endogenously or exogenously produced neurotoxins has been implicated in the progression of this disease. Evidence, however ,is lacking, even though a synthetic neurotoxin, MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), producing a Parkinsonian syndrome in man and animals has been discovered (35).

The presence of lipid content and highly localized large deposits of iron in neurotransmitter-rich brain regions (such as substantia nigra, globus pallidus and caudate nucleus) would make the brain an ideal organ for oxidative stress, resulting from metal induced lipid peroxidation in the presence of H_2O_2 (36). Although the function of regional high brain iron content is not known, the homeostasis of brain iron is thought to be necessary for normal brain function (5,37). Free tissue metal (e.g., Fe^{2+} or Cu^{2+}) can initiate the formation of cytotoxic oxygen free radicals (e.g. hydroxyl radical, OH) from its interaction with H_2O_2 (Fenton reaction) and thus promote the formation of membrane lipid

peroxides. The mobilization of intra cellular and mitochondrial calcium by iron is now thought to be directly involved in cellular toxicity in oxidative stress. Although this phenomenon was originally identified *in vitro*, its *in vivo* occurrence can no longer be questioned. There are numerous examples where iron induced lipid peroxidation and cellular toxicity have been implicated in the pathogenesis of systemic disorders (39). Its application to stroke and trauma-induced neurodegeneration and nigra-striatal dopamine neuron degeneration in Parkinson's disease has not escaped scrutiny (39). These findings highlight the recent reports from a number of laboratories in which a highly selective increase of iron and basal lipid peroxidation and decrease of dismutase activity have been noted in the substantia nigra of Parkinsonian brains (40,41). Furthermore, they are compounded by the decreased ability of Parkinsonian brains to scavenge H_2O_2 due to diminished availability of reduced glutathione (GSH) and glutathione peroxidase activity (40). The latter is the main pathway of H_2O_2 breakdown in the brain.

Both oxidative deamination and auto-oxidation of dopamine results in excessive generation of H_2O_2 The inability to dispose of H_2O_2 could result in H_2O_2 accumulation and its interaction with Fe^{2+} may promote the Fenton reaction.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH$$

Iron-induced oxidative stress and lipid peroxidation can proceed optimally with either Fe^{2+} or Fe^{3+} provided mechanisms exist to facilitate the interconversion of iron between its redox states. Fe^{3+} formed can be converted to Fe^{2+} in the presence of endogenous chelators such as ADP, but ADP is a weak iron chelator and of limited capacity to chelate iron. If, however, we are to implicate iron in the neurodegeneration of substantia nigra it is in this brain region we must look for an endogenous chelator with high affinity for iron. The substantia nigra is unique among the brain regions for having excessive amounts of neuromelanin formed from auto-oxidation of dopamine.

Melanin has the ability to bind high amounts of metals. It is the pigmented melanin containing dopamine neurons which degenerate in Parkinson's disease. Although melanin has the capacity to bind metals, in normal circumstances it is thought to participate in scavenging metal-induced free radicals, wherever it is formed and the best example is the skin. Recently we have demonstrated that melanin has high affinity binding sites for Fe^{3+} (KD = 10 nM) and in this condition it potentiates basal lipid peroxidation in the presence of rat brain cortical preparation (41). The binding of iron to melanin and lipid peroxidation can only be inhibited by a number of iron chelators such as desferrioxamine, U74500A, 8-hydroxyquinoline and chlorpromazine. The potentiation of melanin-iron induced lipid peroxidation has been attributed to the ability of melanin to convert Fe^{3+} to Fe^{2+} in the presence of H_2O_2 and drive a Fenton like reaction with liberation of cytotoxic hydroxyl radical (OH.). This process is thought to take place with Fe^{3+} and not Fe^{2+} (43). The alteration in the ratio of Fe^{2+} to Fe^{3+} from 3:1 in control substantia nigra to 1:1 in Parkinsonian substantia nigra is compatible with induction of lipid peroxidation process (44). This feature has led us to suggest that lipid peroxidative induced stress is a possible phenomenon in the etiology of Parkinson's disease, without involving an endogenously or exogenously derived neurotoxin (5). Accumulation of free tissue iron could act as the neurotoxic agent in Parkinson's disease. However, a number of important questions remain unanswered, namely: (a) how and why does iron accumulate in substantia nigra and why is it further increased in Parkinsonian substantia nigra? (b) what is the mechanism of iron transport into these brain regions low in transferrin receptor? (c) why is it that the regions (globus pallidus, substantia nigra caudate nucleus and dentate gyrus) with high iron content have extremely

low transferrin and transferrin receptor sites? and (d) is iron sequestered and transported from one brain site to another? Preliminary studies using the MPTP induced model of Parkinsonism in mice and monkeys show that the degeneration of substantia nigra does not lead to increase of free tissue iron in this region. The possibility of an infection-induced breakdown of the blood brain barrier in this region, which would allow a greater uptake of iron, cannot be ignored, nor also, the possible genetic basis for the disease. A reduced transport of iron out of the brain may also be contributory since the turnover of iron in the brain is extremely slow. There is no doubt that the problems concerning the metabolism of iron, its deficiency or excess, will occupy important and fertile areas for future research studies.

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IRON DEFICIENCY: LONG-TERM EFFECTS ON LEARNING

Harold H. Sandstead,
Department of Preventive Medicine and Community Health
University of Texas Medical Branch at Galveston, Texas

The possibility that iron deficiency can influence intellectual performance was suggested from clinical observations of patients with hookworm. For example, in 1910, Dock and Bass (1) related that "the intellect is much dulled and mental processes are slow and the patient seems confused and stupid".

In 1919, Waite and Nielsen (2) reported findings on 559 children from Northern Queensland, Australia that related impaired performance on the Binet- Simon, and Porteus maze tests to the severity of hookworm infection. The study showed that performance of the respective tests by lightly infected and heavily infected children was retarded 5.4 and 2.0, and 19.5 and 13.3 months (Figure 1). Degree of retardation was related to the duration of the hookworm infection. Waite and Nielsen acknowledged that Strong (3) had described similar findings in children from North Carolina in 1916, and that according to her report, treatment of hookworm was followed by some recovery of mental function.

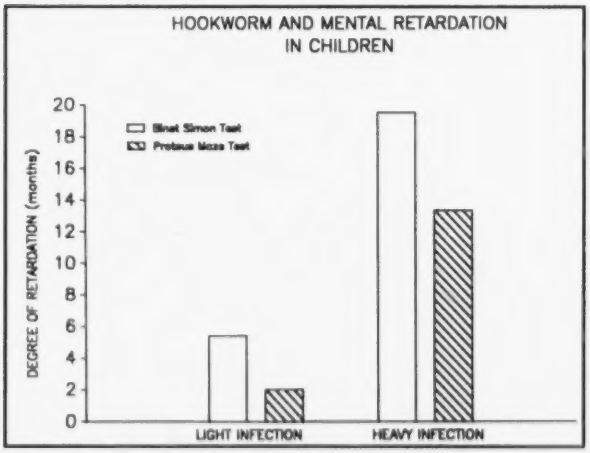

Figure 1. Hookworm infection impaired neurobehavioral function of Australian children (2). The effect was greatest with heavy infection. Presumably iron deficiency was a major factor contributing to the abnormal function.

In more recent times, relationships between brain function and iron nutriture have been studied in infants, children and young adults (4,5). In general, low iron status has been associated with reduced performance on tests of brain function. In some instances, improvements in brain function of infants have not been detected after iron repletion. This finding has prompted speculation that iron deficiency in infants during the postnatal period of rapid brain maturation, can injure the brain and cause behavioral sequelae. This hypothesis is reasonable in view of findings in experimental animal models and known functions of iron in neurochemistry, that are reviewed elsewhere in this symposium. However, for reasons that will be discussed, the current evidence from studies of humans is inadequate to conclude unequivocally that the hypothesis is true. In this review findings that support the hypothesis will be presented, and factors that confound their interpretation discussed. Because of the orientation of this reviewer, the major emphasis will be given to nutritional issues.

Evidence interpreted as strongly supporting the hypothesis was published in 1983 by Palti and colleagues (6). They measured indices of brain function at 24, 36 and 60 months of age in children who attended the Preventive Maternal and Child Health Service of western Jerusalem, and who had had hemoglobin measured at nine months of age. Twenty per cent of the children displayed hemoglobins between 10.9 and 10.0g/dl, while 9.5% had lower levels. Height was significantly ($p<0.04$) associated with

hemoglobin level in males, and marginally so in females (p=0.10). Hemoglobin level at 9 months of age was related to the developmental quotient (DQ) at 24 months of age (p=0.0001, n= 873), the intelligence quotient (IQ) at 36 (p = 0.004, n = 373), and 60 months of age (p = 0.084, n = 230)(Figure 2).

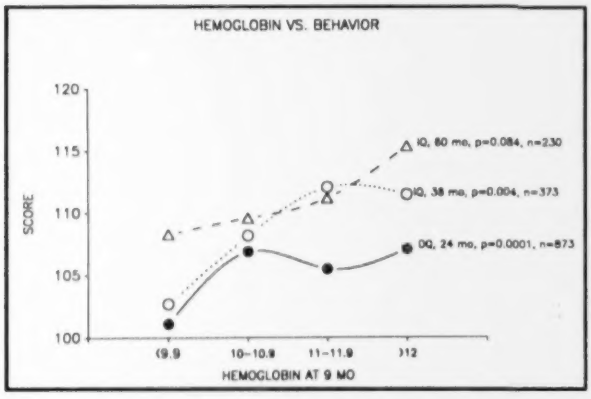

Figure 2: The level of blood hemoglobin at 9 months of age was associated with subsequent brain function of children at 24, 36, and 60 months of age, as measured by developmental quotient (DQ) and intelligence quotient (IQ) (6). The data were analyzed by the analysis of variance.

Effects of independent variables including mother's education, social class, birthweight, hemoglobin and sex on behavioral indices were evaluated (6). The variance explained by the model was 12.8% for DQ at 24 months, 25% for IQ at 36 months, and 37.1% for IQ at 60 months. Mother's education accounted for most of the variance. Hemoglobin level made a significant contribution only at 60 months (p = 0.021, R_2 = 1.8).

To predict the relationship between hemoglobin and brain function, the regression coefficients of the covariates were calculated (6). Other independent variables were held constant, and the relationship between increasing hemoglobin levels and performance was determined. Thus, a 7.0 IQ difference in five-year old children who had hemoglobin levels of 8 and 12g respectively was predicted when birth-weight, sex, and education of the mother were constant (Figure 3). These findings suggest that iron nutriture and/or some factor(s) that are strongly associated with iron nutriture affect the developing human brain in such a way as to influence later function.

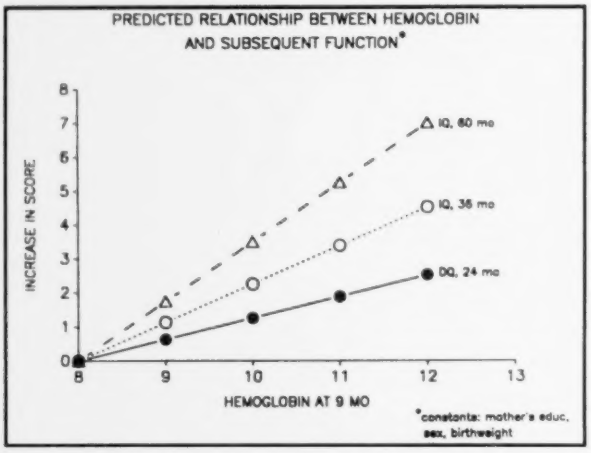

Figure 3: The level of blood hemoglobin at 9 months of age was predictive of subsequent test responses of children at 24, 26, and 60 months for DQ and IQ (6).

Prior to Palti's report, the modern era of careful study of the relationship between iron nutriture and human brain function had been initiated by Webb and Oski (7). They described lower scholastic achievement in iron-deficient adolescents. Their work was prompted by Howell's (8) description of behavioral findings in iron-deficient children that suggested they were less attentive (8).

Subsequently, Oski and Honig (9) found, in a placebo-controlled study, that intramuscular iron treatment rapidly improved the Bayley Mental Development Index (BMDI) scores of North American iron-deficient infants ages 9-26 months. Mean recovery time was 6.8 days (Figure 4).

Oski et al (10) then examined the relationship between BMDI scores and iron status in iron-deficient non-anemic North American infants. At baseline they found lower scores. Iron repletion with intramuscular iron was associated with an increase of about 22

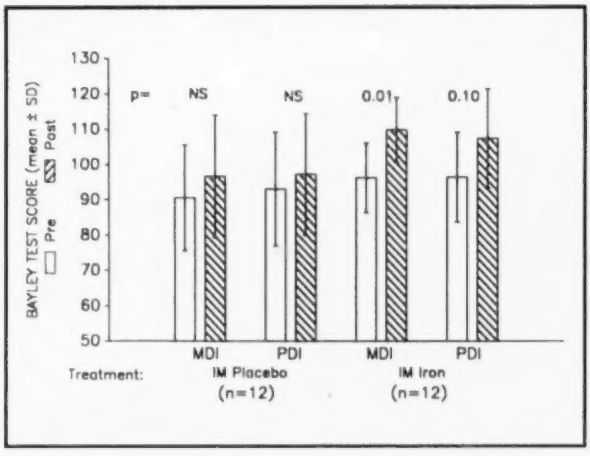

Figure 4. The Bayley Mental Development Index scores of anemic North American infants responded to intramuscular iron therapy in about seven days (9).

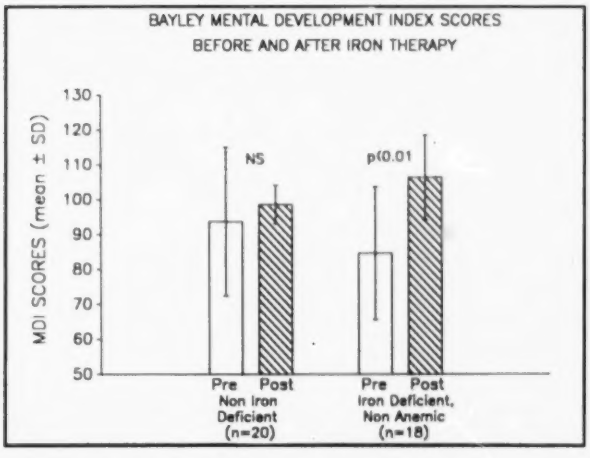

Figure 5. The Bayley Mental Development Index scores of non-anemic-iron-deficient North American infants responded to intramuscular iron therapy in about seven days (10).

points in the MDI (Mental Development Index) score of the 18 iron-deficient infants in seven days. In contrast, the 10 iron-depleted and 10 iron-adequate infants displayed a non-significant increase in the MDI (Figure 5). Oski et al (10) also found that 4 of 20 infants in the non-iron-deficient group displayed an MDI increase of at least 10 points, while in the iron-deficient non-anemic group, 14 of 18 infants displayed such an increase (X_2 = $p<0.001$). As will be seen, these findings of Oski were not the same as those in iron-deficient infants from less developed countries.

While Oski was working in the United States, Lozoff et al (11) studied Guatemalan anemic infants, ages 6-24 months. They found low BMDI scores in anemic iron-deficient infants. However, in contrast to Oski's studies, Lozoff did not find an improvement in test scores after six days of repletion with oral iron, even though hemoglobin increased appropriately (Figure 6).

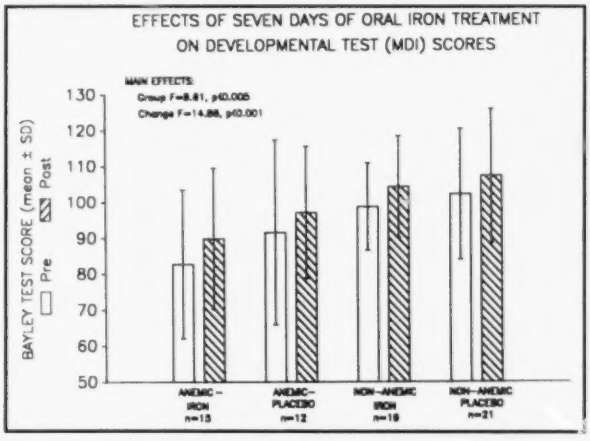

Figure 6. The Bayley Mental Development Index scores of Central American anemic infants did not respond significantly to seven days of oral iron treatment (11).

Lozoff et al (12) examined their data for relationships between age and the severity of iron deficiency and the presence of developmental defects. They found that the older iron-deficient infants had the lowest MDI scores. In 27 infants ages 19-24 months, "the more severe the iron deficiency the more severe the developmental defect". The correlation between severity of iron deficiency and MDI scores was 0.73 ($p < 0.001$). Anthropometric indices suggested that the nutrition status of the anemic infants was inferior to the control group.

More recently Lozoff et al (13) reported ob-

servations on 191 Costa Rican infants ages 12 to 23 months. Iron-deficiency-anemia was associated with lower BMDI scores, as compared to infants with iron deficiency without anemia, or iron-replete infants. Following three months of oral iron repletion, 18 of 51 anemic-iron-deficient infants became fully iron-sufficient. While their MDI scores did not show significant improvement, their psychomotor development index (PDI) scores increased about 10 points ($p = 0.007$) to a level similar to that of the controls. In contrast, the 30 infants who were no longer anemic but not fully iron-sufficient, continued to have PDI scores that were lower than the controls. The reason why this group did not achieve full restoration of iron nutriture is unclear. The limited dietary information reported suggests that other nutrient deficiencies were present. Consistent with the presence of such confounding variables was the finding that less than 15% of variance in the BMDI scores was accounted for.

Subsequently, Lozoff [14] discussed her findings at a symposium sponsored by the World Health Organization (WHO). She reported that the Infant Behavior Record (IBR) from the BMDI showed that a greater proportion of the anemic infants in her Costa Rican study (13) had displayed abnormal affective behaviors when compared to the non-anemic group (36% vs. 13%, $p < 0.05$) and that orientation was similar between the two groups. Abnormal affective behaviors were closely related to the poor mental scores of the anemic iron-deficient infants. Regardless of age, anemic infants with abnormal affect scored substantially lower on the MDI than anemic infants who had normal affect. This latter group had MDI scores that were similar to those of non-anemic infants. This suggested that "poor developmental test performance may not be a consistent manifestation of iron deficiency in infants, but rather may be observed only in a sub-set of babies who display behavioral disturbances". Consistent with this suggestion, was an association between improvement in affect and improvement in test scores, which was independent of treatment.

Mother-infant interactions did not account for findings in the Costa Rican study (14). It seemed likely that other unidentified environmental factors influenced the outcome of her study because only the fully iron-repleted infants showed significant improvement in BMDI scores (13). Those who continued to have iron deficiency, continued to have lower BMDI scores. The failure of these infants to become fully iron-replete in three months suggested that other unidentified factors had inhibited their iron utilization and perhaps their BMDI scores. Lozoff (14) did find that the child-rearing environment was inferior in the group that was less responsive to iron supplements. Maternal IQ was related to the hemoglobin levels in the infants ($p = 0.0002$), and anemia was inversely related to the level of stimulation received ($p = 0.001$). Conversely, maternal IQ and stimulation did not account for the MDI or PDI scores of the infants.

Because it is generally true that humans are seldom deficient in only one nutrient when deficiency is related to diet, it seems likely that Lozoff's subjects were deficient in other nutrients besides iron. Because this possibility was not investigated, one can only speculate as to the deficiencies that were likely present. A nutrient which is a prime candidate for investigation as a confounding variable in studies such as those described above is zinc. Iron and zinc are present in substantial amounts in many of the same foods, and dietary factors that inhibit absorption of iron, often inhibit absorption of zinc. The phytate present in foods is one of the important inhibitors of iron and zinc retention (15,16,17). In addition, iron and zinc are both poorly bioavailable from cow milk, because of its low iron and zinc content, and because of the formation of insoluble complexes of iron and zinc with calcium and the phosphopeptides of casein that are produced from tryptic digestion (18). Clinically, children with severe iron defi-

ciency from parasitic infections such as hookworm often display substantial growth retardation (19,20), which is now known to be caused, at least in part, by zinc deficiency (21).

Zinc is known from studies in animals (22) and humans (23) to be essential for development and function of the brain. What is poorly defined in humans is the relationship of level of zinc nutriture to brain function. Research is needed to determine if low zinc nutriture is partly responsible for findings in infants such as those described by Lozoff (11-14).

The complexity of this research question is further illustrated by studies in which Walter et al (24) evaluated 196 Chilean infants. Hemoglobin was measured at 9 months of age; at 12 months of age, iron status and BMDI scores were determined and the infants treated with oral iron for 90 days. Thirty-nine anemic-iron-deficient infants had lower BMDI scores ($p<0.001$) than the 127 non-anemic-iron-deficient infants or 30 iron-sufficient infants, which had similar scores. The BMDI scores were poorly responsive to iron repletion, even though anemia was corrected. Infants who had been anemic for more than three months, had lower BMDI scores than those who had been anemic for less than three months ($p<0.05$). Factors responsible for the non-responsiveness of the BMDI scores were not identified.

Subsequently, Walter (25) discussed his findings at the above noted symposium. A surprising find was that decrements in MDI score occurred over a fairly narrow range of hemoglobin (from 11.0 to 10.5g/dl). Because none of the infants in Walter's (25) study showed an improvement in BMDI performance following iron repletion, it is difficult to conclude that iron deficiency was unequivocally the cause of his findings. Usually the final proof of a nutrient deficiency's involvement in a physiologic abnormality is that the affected subjects respond to treatment. As noted by Professor Oski (26) in his discussion of Walter's (25) paper, unidentified nutritional deficiencies, toxicities or other confounding variables that might have also impaired the brain function of the infants, or inhibited their response to iron were not evaluated.

Consideration of future research approaches seems appropriate. Other putative nutritional deficiencies or toxicities should be assessed by chemical analysis of index tissues and by controlled therapeutic trials.

Intervention trials should include at least three study groups: putative deficient nutrient plus placebo, putative deficient nutrient plus nutrient supplement, and placebo. Other groups which would provide additional control include nutrient supplement, and an observation group that receives no treatment. The nutrient supplement should probably provide about 50% of the recommended dietary allowance (RDA) and the mean safe and adequate amount of nutrients, for which an RDA has not been identified (27). A possible exception is folic acid. Consideration should be given to supplementing folate at 25% of the RDA because of evidence that monoglutamate folate can interfere with zinc retention in some humans (28). If it appears that the indigenous diet of the subjects is likely to be deficient in certain amino acids or energy, modest amounts of these should also be included in the nutrient supplement. This approach should decrease the likelihood that unidentified nutritional deficiencies confound the response of the subjects to treatment with the nutrient in question. It is clear that unless all nutritional possibilities are controlled for, it will be difficult to conclude that a specific nutrient is responsible for inhibition of the physiologic responses that are indicative of brain function.

Other approaches for evaluation of brain function might be useful. Pollitt (29) has questioned the use of the BMDI for evaluation of particular brain functions. Tests that more closely reflect cognition would seem appropriate. Another approach that might be useful is measurement of electrophysiologic responses of the brain to stimuli.

Tucker et al (5) used this approach in studies of relationships between body iron stores of college students and brain function.

In summary, published evidence supports the hypothesis that iron deficiency during early post-natal life in humans can injure the brain and result in neurobehavioral sequelae. The evidence is not, however, conclusive. Future research must attempt to control confounding variables that prior knowledge suggests are likely to be present.

Abstract

Iron is known to be essential for brain function. Research in human infants suggests that iron deficiency early in postnatal life can result in sequelae that impair cognitive function. This interpretation is reasonable because it is consistent with findings from well-controlled experimental studies in animals. On the other hand, nutritional deficiencies related to diet seldom occur singly in humans. Therefore it is possible that deficiencies of other nutrients which are associated with iron in food contribute to the findings in human infants. Future research might profitably evaluate the roles of other nutrients and utilize other methods for measurement of brain function.

Acknowledgements

The figures used in this paper are derived from published data in the cited papers. To our knowledge, these data have not been published as figures previously. The author thanks the primary authors of the data for their lucid presentations, and the organizers of this symposia for the opportunity to review this topic.

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IRON DEFICIENCY AND CHILDHOOD LEAD POISONING

Ray Yip,
Division of Nutrition
Center for Chronic Disease Prevention
and Health Promotion
Centers for Disease Control
Atlanta, Georgia 30333, USA

Several links have been noted between iron deficiency and childhood lead poisoning. There is a greater prevalence of both conditions among children of lower socioeconomic status (SES), and several clinical studies have found high prevalence of iron deficiency among children with lead poisoning (1-5). One hypothesis to explain this association is that iron deficiency and lead poisoning are independent except for sharing a simple risk factor—poverty. Poverty not only leads to poor nutrition, and thus to a greater risk for iron deficiency; it also leads to poor housing with increased risk for environmental lead exposure. A second, more interesting hypothesis is a causal relationship in which iron deficiency enhances lead poisoning.

The nutrition and biochemistry data collected by the United States Second National Health and Nutrition Examination, 1976-1980 (NHANES II), conducted by the National Center for Health Statistics provided an opportunity to take a closer and more complete look at the relationship between childhood iron deficiency and lead poisoning (6). Unlike clinical studies, which only examine the iron status of children diagnosed for lead poisoning, NHANES II has complete information on both iron and lead status for a representative sample of the U.S. children. Besides permitting the examination of iron-lead interaction from a socioeconomic and epidemiological perspective, the NHANES II data also provide valuable clues on the laboratory interactions of iron and lead that have significant implications for the assessment of iron as well as lead status.

The NHANES II Sample

A total of 27,801 persons from 64 sampling areas were selected in a probability sample designed to be representative of the U.S. population aged 6 months to 74 years (6). This study included only 1,148 children aged 1 to 5 years who had complete iron biochemistry tests, hematologic tests, and blood-lead levels. All statistical analyses were weighted to reflect the probability of sample selection.

Laboratory Analyses

The biochemical analyses used for this study were performed by the Division of Environmental Health Laboratory Sciences, Center for Environmental Health and Injury Control, CDC. Erythrocyte protoporphyrin (EP) was measured with a modified extraction method of Sassa and Garnick (7). The serum iron (Fe) and total iron binding capacity (TIBC) were measured by the automated Technicon AAII-25 method (8). Whole blood lead (Pb) was measured by atomic absorption spectroscopy (9). All hematologic tests were performed at the examination site. Hematocrit was measured by using the standard microcentrifuge method, and red cell number was measured by using an electronic cell counter. The mean cell volume (MCV) was calculated by dividing the microhematocrit value by the red cell number.

Definition of Iron Deficiency and Lead Poisoning

The EP test is commonly used as one of multiple tests to characterize iron deficiency. However, because EP also responds to lead poisoning, it was not used in this study to

define iron deficiency. For purposes of this study, the following definitions were used:

1. Iron Deficiency—defined as either a low MCV or a low transferrin saturation (Fe/TIBC). Age-specific cutoffs for MCV and Fe/TIBC were: age <2 years, MCV <70 and Fe/TIBC <10%; age 24-60 months, MCV <73 and Fe/TIBC <12% (6).
2. Elevated Blood Lead—defined as whole blood lead > 25 ug/dl (based on CDC recommendations) (10).
3. Elevated EP—defined as 35 ug/dl of whole blood (10).
4. Lead Toxicity—defined as both elevated blood lead and elevated EP according to recommendation from CDC (7).

The Socioeconomic Interaction of Iron and Lead, NHANES II

Children of lower SES are known to be at greater risk for both iron deficiency and lead poisoning. The NHANES II has complete information on SES, iron nutrition status, and blood-lead status, which permitted the characterization of their relationship based on the same cohort of children. Figure 1 shows the prevalence of iron deficiency and elevated blood lead across four SES groups based on family incomes.

This figure clearly demonstrates that low SES is associated with increased risks of both childhood iron deficiency and lead poisoning. In fact, the estimated prevalence of elevated blood lead among the lowest SES group is almost six to seven times that of the highest SES group. The association of iron deficiency and lead poisoning with low SES does not tell us, however, whether low SES is a common risk factor for both conditions, or whether one condition predisposes a child to the other condition.

The Epidemiological Interaction of Iron and Lead, NHANES II

One way to take a closer look at the iron-lead interaction observed among children of lower SES is to see whether the children who are iron deficient also have a greater prevalence of elevated blood-lead. To examine this, the prevalence of elevated blood-lead levels of NHANES II children were compared by grouping three levels of iron nutrition status: (1) normal (normal Fe/TIBC and normal MCV); (2) intermediate (either low Fe/TIBC or low MCV); and (3) iron deficient (low Fe/TIBC and Low MCV) (Figure 2). Additionally, to determine whether children with elevated blood lead were more likely to be iron deficient, the prevalence of iron deficiency among four groups based on blood-lead status were compared (Figure 3).

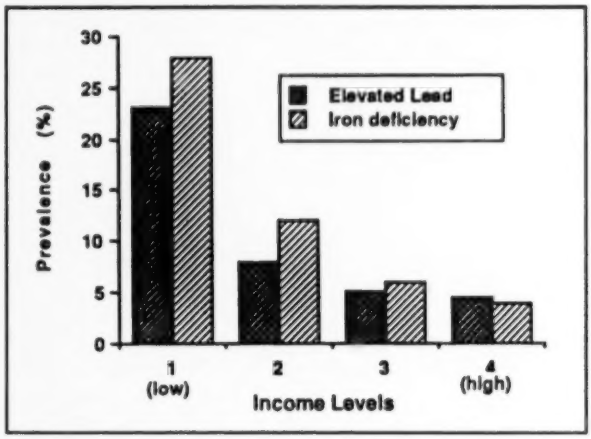

Figure 1: Prevalence of iron deficiency (low Fe/TIBC and/or low MCV) and elevated blood-lead (Pb >25 ug/dl) among four income groups. The income grouping was based on the poverty index which divides household income by number of individuals in a household.

When the group with normal iron nutrition status was used as the reference group, children in the intermediate group had a 40% increased risk of having an elevated blood-lead level. For the iron deficient group, the risk was increased by 340%. This finding indicates that the high prevalence of both iron deficiency and lead poisoning observed among children of lower SES appears to be due to an interaction between the two conditions.

The epidemiologic interaction of iron and lead observed among NHANES II children

confirms and expands the previously observed iron-lead interaction in clinical settings. These studies have shown that children with lead poisoning are often also iron deficient (2-5). The clinical and epidemiological interactions of iron and lead suggest the possibility that either iron deficiency predisposes a child to lead poisoning or vice versa. A series of experiments that studied the physiologic interaction of iron and lead have helped to further define the nature of the observed association between childhood iron deficiency and lead poisoning.

The Physiological Interaction from Previous Studies

By far the most important information supporting a causal relationship between childhood iron deficiency and lead poisoning is a series of controlled experiments of lead absorption among animals and humans (3). Animal studies show that iron deficiency increases lead absorption (11,12) and tissue retention of lead (lead toxicity) (12,13). Human studies show that persons with iron deficiency absorb more lead than those who are iron sufficient (14). These studies provide the critical link in defining the nature of the association between iron deficiency and lead poisoning. The clinical and epidemiological iron-lead interaction can be explained, at least in part, by the increased lead absorption and lead toxicity among children with iron deficiency. The evidence taken together suggests that childhood iron deficiency contributes significantly to lead poisoning.

The Behavioral Interaction of Iron and Lead

Besides the physiological interaction of iron and lead, which helps to establish a causal relationship between iron deficiency and lead poisoning, a behavioral interaction of iron and lead may also contribute to the relationship between the two. Pica, the compulsive ingestion of unusual or nonfood items, has been shown to be associated with iron deficiency among adults and children (15-17). Often the pica behavior ceases with iron treatment (17,18). For younger children living in households with lead dust, even normal hand-to-mouth activity would pose a risk for lead ingestion from paint chips, dirt, and dust, and pica behavior would likely increase lead ingestion (2). If pica is indeed a consequence of iron deficiency, it should be considered as part of the adverse neuropsychological consequences of iron deficiency. Both iron deficiency and lead poisoning have also been well documented to have adverse effects on child behavior and development (19,20). Further study is needed to determine whether iron deficiency and lead poisoning interact at the central nervous system level, that is, does iron deficiency enhance the neurotoxicity of lead?

The Significance of Laboratory Interaction of Iron and Lead

Besides providing a complete picture of the socioeconomic and the epidemiological interactions of childhood iron deficiency and lead poisoning, NHANES II also is a unique source to better define the relationship of EP with both iron and lead. The EP test has been used alone as a screening test for iron

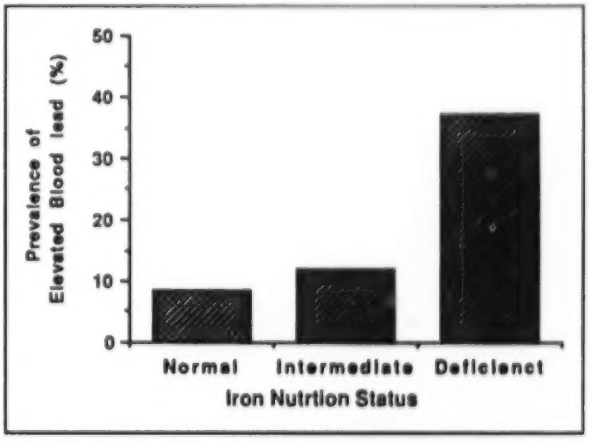

Figure 2: Comparison of prevalence of elevated blood lead (Pb >25 ug/dl) at three different levels of iron nutrition status. Three levels of iron nutrition status are: (1) normal (normal Fe/TIBC and normal MCV); (2) intermediate (either low Fe/TIBC or low MCV); and (3) iron deficient (low Fe/TIBC and Low MCV)

deficiency and used with serum ferritin and transferrin saturation in characterizing the iron nutrition status (21,6). The EP test is widely used in the United States for screening of childhood lead poisoning and for classifying the severity of lead toxicity (10). However, the relative contributions of iron and lead status to EP elevation have not been fully assessed. Table 1 illustrates the influence of both iron and lead status on the mean EP level. The groupings for iron nutrition status were based on the combined results of MCV and Fe/TIBC as detailed in Figure 2.

Table 1. Mean erythrocyte protoporphyrin values at varying status of iron nutrition and blood-lead. Iron nutrition status is based on the combination of Fe/TIBC and MCV test results

	Iron Nutrition Status		
Blood Lead (ug/dl)	Normal	Intermediate	Deficient
<10	20.2	21.9	-
10-24	20.9	22.6	40.0
25-40	29.8	31.7	73.8
>40	54.7	54.4	107.0

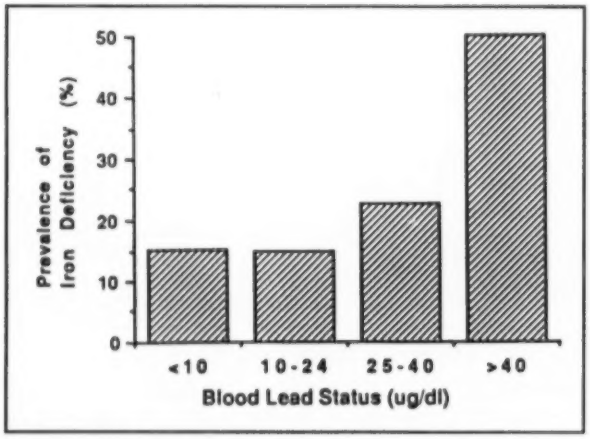

Figure 3. Comparison of prevalence of iron deficiency at four levels of lead status.

The results in Table 1 indicate that when the blood-lead level exceeds 25 ug/dl and when iron deficiency is present (both MCV and Fe/TIBC are low), mean EP value departs from the baseline (normal iron nutrition status and low blood-lead level). Elevated blood-lead and iron deficiency appear to have an additive effect on EP. These findings indicate that an elevated EP value can be caused by iron deficiency, elevated blood-lead, or both. Therefore, it is proper to take both iron and lead status into account while using EP as a screening or diagnostic test for either iron deficiency or lead poisoning.

The socioeconomic and epidemiologic interactions of iron deficiency and childhood lead poisoning observed from NHANES II, combined with experimental evidence, help to define the relationship between iron deficiency and lead poisoning. Poverty is a common risk factor for both conditions, since children of lower SES are more likely to live in substandard housing with increased exposure to lead paint. Such children are also at increased risk for iron deficiency (increased host vulnerability). Evidence thus far indicates that children with an iron deficiency who are living an in at-risk environment may have several factors that can further increase their risk of lead poisoning. These include behavioral factors that can increase lead ingestion (pica), increased absorption of ingested lead, and possibly, increased tissue retention (toxicity). For these reasons, childhood lead poisoning should be considered as one of the many adverse consequences of iron deficiency. More importantly, this strong interactive iron-lead relationship emphasizes the importance of improving childhood iron nutrition status to help prevent childhood lead poisoning. Other implications from the study of iron-lead interactions are: (1) the need to consider both iron and lead status when using EP test; (2) because both iron deficiency and lead poisoning affect child behavior and development, it is important to control for lead status when studying the effects of iron on the brain, and vice versa; and (3) in the treatment of lead poisoning, routine iron supplementation should be considered.

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IRON DEFICIENCY - RESEARCH PRIORITIES

Ray Yip,
Division of Nutrition
Center for Chronic Disease Prevention and
Health Promotion,
Centers for Disease Control
Atlanta, Georgia 30333

Introduction

Iron deficiency is the best studied of all the nutritional deficiencies. This is because iron deficiency is relatively common and also because of the availability of an excellent biological marker - red blood cells - for the assessment of iron status. The ease of hemoglobin or hematocrit testing makes detection of iron deficiency anemia relatively feasible. Although there are many causes of anemia, it is well accepted that iron deficiency is the most common cause (1,2). In fact, in many settings, the terms "anemia", "iron deficiency" , and "iron deficiency anemia" are used interchangeably. In recent years, several biochemical tests such as transferrin saturation, serum ferritin, and erythrocyte protoporphyrin have become available and they have enhanced our ability to diagnose iron deficiency beyond anemia. Transferrin receptor testing, now on the horizon, likely will join the list of tests which can help define iron nutrition status. Because of the multiple tests at our disposal in studying iron deficiency, we have learned a great deal about this nutritional disorder. However, in part because of this great knowledge base, we find a number of issues ahead of us to be addressed, perhaps akin to the phenomenon of the more we know, the less certain we are.

Based on knowledge of various aspects of iron deficiency, the following is a series of research questions that need to be addressed in the next decade. It is also my bias that most of these questions can be addressed within the proposed time frame. This proposed list is by no means exhaustive, rather it reflects my view on this field.

1. Issues related to using hemoglobin and hematocrit as screening for iron deficiency

Hemoglobin and hematocrit measurements are by far the most commonly performed clinical tests for the purpose of detecting anemia. For practical purpose, anemia found in women and children is regarded as iron deficiency anemia. In the definition of hemoglobin cutoff points for anemia, a normal variation of hemoglobin between children and adult, and between women and men, is well accepted. It is also recognized that residence at higher altitude and cigarette smoking result in higher hemoglobin levels, requiring different cutoff points (3). One unresolved issue is:

Are separate criteria needed for blacks and whites?

Many studies comparing the mean Hb between blacks and whites consistently find that blacks have significantly lower Hb level than whites. It appears that the difference is about 0.5 to 0.8 g/dl of Hb. Because of this persistent finding, many have proposed the adoption of a lower Hb cutoff value to define anemia (4). However, this is not a step to undertake without careful evaluation of the impact of such decision. Will lowering the anemia cutoff point for blacks result in less ability to detect iron deficiency among blacks? In order to fully study this race-specific hemoglobin cutoff issue, the following question needs to be answered:

What is the nature of lower hemoglobin levels among blacks?

If the lower hemoglobin among blacks is truly due to a generalized "physiologic" variation between races, then separate cut-

offs can be considered. On the other hand, if it is due to a subpopulation of blacks who have restricted red cell production such as alpha- or beta-thalassemia traits, then it would not be appropriate to use lower cutoffs to accommodate these genetically mediated mild anemias. Thus far, no study compares hemoglobin levels between blacks and whites after adjusting for nutrition status as well as after excluding individuals with mild hemoglobin production deficits. Indirect evidence - the high prevalence of mild alpha-thalassermia among blacks (30% with one alpha gene deletion) and greater variance of black Hb distribution - does not support the need for adopting race-specific Hb cutoffs for anemia.

What is the normal range of hemoglobin values throughout pregnancy?

Many studies have observed that hemoglobin level declines early in pregnancy, most likely due to plasma volume expansion. If adequate iron nutrition is provided, the hemoglobin would reach the lowest level by the end of second trimester and gradually rise during the third trimester (3). The magnitude of Hb changes observed during pregnancy is no less than the difference of Hb between men and women. It appears that a gestation-specific Hb cutoff point would better serve the diagnosis of anemia of pregnancy. So far, there are no available hemoglobin data based on healthy iron supplemented pregnant North American women that can be used to define anemia in pregnancy. In addition, to properly define the normal range of hemoglobin during pregnancy, the normal range of iron biochemistry tests, including transferrin saturation, serum ferritin, and erythrocyte protoporphyrin, need to be defined also. Evidence suggests that these biochemical tests undergo significant change throughout pregnancy among healthy iron-supplemented pregnant women.

What is the meaning of anemia of the elderly? Is it physiologic or pathologic?

It has been well observed from large surveys that mean Hb levels decline gradually after 50 years of age with concomitant increase in the prevalence of mild anemia (1). It has been proposed that this may be an aging phenomenon, slowing down of hematopoiesis with advancing age. If such is the case, a lowering of Hb criteria for elderly individuals would be appropriate. Recent study from the First National Health and Nutrition Examination Survey 1970-1975, found that many of the elderly with anemia show evidence of mild but significant inflammatory conditions (elevated erythrocyte sedimentation rate)(5). Therefore, mild pathological conditions appear to be playing a role in the "anemia of elderly". However, in the same study population only about half the hemoglobin reduction observed can be attributed to inflammation. This leaves open the possibility that other factors including normal aging process may be playing a role in the reduction of Hb level. This is an issue that can be potentially resolved by a cross-sectional study of the elderly with detail characterization of their health and nutrition status.

2. Detection of iron deficiency: beyond anemia screening

The most complete clinical assessment of iron deficiency requires the use of multiple hematologic and biochemistry tests. It is well accepted that among the spectrum of the iron deficient state, iron deficiency anemia represents the relatively more severe cases and iron deficiency without anemia represents the milder cases. The reliance on anemia as a screening index would miss those individuals with biochemical evidence of iron deficiency yet without anemia. This problem is further compounded by the fact that iron deficiency anemia among younger children has been declining in the U.S. and the remaining cases appear to be milder (6,7). Even though the improvement of iron nutrition status of

younger children is an encouraging phenomenon, the resulting change of relationship between anemia and iron deficiency may require some revision in our approach to screening of iron deficiency. This is because as iron deficiency and anemia become less common, the remaining cases of anemia are more likely related to other causes. In other words, Hb and Hct are becoming a less efficient tool for screening of iron deficiency. In such circumstances, the logical question is:

How to improve the clinical screening and diagnosis of iron deficiency?

Among the major biochemical tests - transferrin saturation, serum ferritin, and erythrocyte protoporphyrin, only erythrocyte protoporphyrin test has been adapted into a simplified outpatient procedure (hematofluorometry). However, this procedure still lacks proper standardization and quality control procedure. Further effort is needed to make erythrocyte protoporphyrin a valid screening test for iron deficiency. Serum ferritin probably offers the best specificity in detecting iron deficiency and represents the current test of choice among the three major tests. However, without the adoption of serum ferritin into a simplified (low cost and easy to perform) outpatient screening test , it is unlikely to become a widely used test for general screening. Therefore, from the laboratory development point of view, improvement of erythrocyte protoporphyrin method and simplification of serum ferritin method hold great promise for improving the screening and diagnosis of iron deficiency

3. Beyond early screening and treatment for iron deficiency: the emphasis of prevention as the primary approach

A. Infancy and early childhood

One of the greatest accomplishments in the study of iron nutrition is the determination that the reasons for the high incidence of iron deficiency anemia in childhood is related to inadequate iron intake in infancy. This has resulted in the iron supplementation of infant diet and the effort to improve infant feeding practice which in turn have led to a significant reduction in childhood iron deficiency anemia. Studies have established that the early use of whole milk and formula without iron contribute to the development of iron deficiency, whereas breast feeding and the use of formula with iron appear to be protective (8-9). These findings have become the cornerstone for the changes in recommendations of infant feeding practice (10-11). The gradual changes in infant feeding practice and the increase in iron content of the diet of infants and younger children can be viewed as an effective program in the prevention of iron deficiency. One major remaining question left on the prevention of childhood iron deficiency is:

How long do infants need to receive iron supplement to avoid iron deficiency?

More specifically, the key research question is:

Is infant feeding up to 6-months of age with iron-fortified formula adequate for prevention of iron deficiency in early childhood?

There is little doubt that iron supplementation or fortification of infant diet is a sound prevention strategy. Even though a majority of U.S. infants start on breast feeding, by 3-months of age only a minority of them remain breast fed. The existing recommendation for non-breast fed infants is use of iron-fortified formula up to either 6 or 12 months of age. There are well conducted studies to show that the use of iron fortified formula up to 12 months of age protects against iron deficiency (12), but there is lack of evidence that the use of formula with iron to only 6-months of age provides an adequate protection against iron deficiency. Despite the lack of evidence, the American Academy of Pediatrics recommends use of iron-fortified formula to 6-months of age with qualification (13). It is evident that a carefully conducted clinical trial is needed to resolve the proper duration of iron supplementation in infancy.

Faced with the uncertainty of the answer to above questions, major public nutrition programs such as WIC (Special Supplemental Food Program for Women, Infants, and Children) continue to provide iron fortified infant formula up to 12 months of age to eligible non-breast feeding infants. Because of the economic implication in the length of formula use, this is an important question in infant iron nutrition.

B. Women of child bearing age

Women of child bearing age represent the other major group at risk for iron deficiency. The risk appears to be even higher during pregnancy because of increased iron need compounded by marginal pre-pregnancy iron status. Although anemia screening and iron treatment are the standard practices, it would be preferable to prevent iron deficiency. Unlike infancy, for which specific items such as formula and cereal can be fortified with iron, there is a lack of specific dietary items for women which could be used as vehicles for iron fortification. The most suitable general food vehicle for iron fortification in western countries is flour. However, the major concern for iron fortification of flour is the possibility that individuals with certain conditions are at risk for iron overload such as those with hereditary hemachromatosis and those with severe hemolytic anemia would suffer further damage from extra iron in the diet. However, it is not clear what would be the extent of harm to those at risk for iron overload if more iron is added to the flour. Thus far, among developed countries, only Sweden has adopted enhanced iron fortification of flour for more than a decade and a significant surge of significant iron overload has not been observed. Currently, U.S. flour is partially iron-fortified. In order to prevent iron deficiency for adult women greater level of iron in flour is needed. To do so, the following question needs to be addressed:

What is the risk or benefit of increased iron content in food?

One potential approach to this difficult question is to take a closer look at the Swedish experience. How much reduction in iron deficiency among adult women versus how much increase in morbidity and mortality related to iron overload has been observed?

Perhaps another approach to this issue is to consider the development of effective screening programs for individuals at risk for clinical iron overload. It is possible that treatment for individuals at risk for iron overload can allow the individual at risk for iron deficiency to benefit from the prevention program. The merit of this proposed approach is that many of those individuals at risk for iron overload would develop clinical complications even if the diet were not iron-fortified A screening program for iron overload can potentially benefit these individuals regardless of the iron content in the flour. A research question related to this contention is:

Would identification and treatment of individuals at-risk for iron overload reduce their risk of morbidity and mortality?

If the answer is affirmative, it may open the door for improvement in the iron deficiency prevention program for women.

4. Define the functional significance of iron deficiency.

(A). Relation to child behavior and development

Among the adverse consequences of iron deficiency other than anemia, effects on child behavior and development are the best studied areas. Recent evidence by Lozoff, et al from Costa Rica and by Walter, et al from Chile indicate that infants with iron deficiency anemia have significantly lower developmental testing results than normal infants (14,15). Even more disturbing is that children who had iron deficiency anemia during infancy were still found to have deficit in development years after correction of iron deficiency (Walter, personal communication). Even though the two most recent

studies strongly suggest that iron deficiency anemia results in adverse child development, these studies did not have the sensitivity to establish a causal relationship. Therefore, the next task is to conduct similar investigations with improved design to properly determine the presence or absence of causal relationships. The general research question for this endeavor is:

Does iron deficiency anemia truly cause impairment in child behavior and development?

The studies by Lozoff and Walter found iron deficiency anemia associated with impaired child development, but did not find an association with "iron deficiency without anemia" (14,15). If a more sensitive instrument can be used to measure behavior and development, and if the study sample size can be increased, the following question deserves another look also:

Does iron deficiency without anemia result in impaired child behavior and development?

(B). Determine the presence and the physiological basis of reported symptoms of tiredness, fatigue, and pica behavior related to iron deficiency in the adult.

It is well accepted among many clinicians that tiredness and fatigue are common symptoms in women with iron deficiency anemia. It would be helpful , using more objective psychological and neurological testing methods, to establish the relationships of these symptoms with iron deficiency. In other words, **Does iron deficiency or iron deficiency anemia result in tiredness and fatigue in adult women?**

One phenomenon, pica, has been reported repeatedly to be observed among women with significant iron deficiency and subsides upon iron treatment. Even though, like tiredness and fatigue, pica is not firmly established as a "true" consequence of iron deficiency, the uncommon nature of this behavior makes it more likely to be the case. If so, the logical question to ask is:

What is the neurobiochemical basis for the symptoms and altered behaviors related to iron deficiency?

C. Defining the relationship between anemia, iron deficiency in pregnancy and adverse birth outcome.

A number of studies have implicated anemia during pregnancy in increased fetal death, premature birth, and low birth weight birth. Most of these studies are ecological in nature and it is difficult to determine the exact relationship between anemia and adverse birth outcome. The nature of anemia during pregnancy is not clear either, even though there is little doubt that iron deficiency plays a significant role. The normal practice of prenatal care is to provide iron supplementation together with other minerals and vitamins. Thus far there are no systematic evaluations of either the impact of iron deficiency during pregnancy or the need for iron supplements. Two research questions deserve attention:

(a). Does iron deficiency or iron deficiency anemia result in adverse birth outcome?

(b). Does iron supplementation during pregnancy reduce adverse pregnancy outcome?

5. Issues related to iron deficiency in developing country settings

Unlike the case of developed countries where other nutrient deficiencies are relatively rare, in many developing countries iron deficiency often coexists with other nutrient deficiencies. In settings where multiple nutrient deficiencies are common, the interpretation of the cause of anemia as well as the assessment of hemoglobin response to iron treatment is far more difficult than when iron deficiency is the only problem. Aside from the complication of other nutrient deficiencies, the higher incidence of infections further renders the assessment of iron nutri-

tion status difficult. This is because inflammatory response to any significant infection (viral, bacterial, parasitic, and malaria) can alter iron metabolism and reduce red cell production. For example, the presence of inflammation would render serum ferritin value higher and would complicate the diagnosis of iron deficiency. Based on our knowledge, it is fair to conclude that, in the developing country setting, screening and diagnosis of iron deficiency is far more difficult than in the developed countries. Several questions pertaining to this issue include:

What is the meaning or cause of severe anemia in developing counties?

Because of the potential multiple causes of anemia, it would be helpful to determine the role of iron deficiency in the contribution to severe anemia in malaria-endemic areas. Even though severe anemia is common in many tropical countries and often attributed to malaria, the exact relationship between severe anemia and malaria is not clear. There is an increasing concern for severe anemia because of the increasing risk of HIV (human immunodeficiency virus) infection from blood transfusion, the common therapy of severe anemia. It is time to take a closer look at the need for transfusion as well as the underlining reason for severe anemia. One aspect of this issue is:

Does iron deficiency contribute significantly to the development of severe anemia related to malaria?

In addition to infection and malaria, other nutrient deficiencies that occur in the developing country settings can complicate the assessment of iron nutrition status. Other than the well known mixed anemia of combined iron and folate deficiency, vitamin C deficiency appears to result in anemia through alteration in iron metabolism. Also vitamin A deficiency appears to alter response to iron treatment for iron deficiency. It is possible that any major disturbance to normal physiological function would alter iron metabolism. If this is the case, then the challenge facing us is:

How to assess iron nutrition status when faced with other physiological stresses?

If we can develop improved methods to diagnose and treat iron deficiency in the difficult and complex environment of developing countries , it will greatly improve our ability to deal with this common nutrition problem in the U.S.

Summary

The series of research questions outlined represent a group of challenging activities that will be helpful in the control and prevention of iron deficiency. For workers interested in iron nutrition, we should feel fortunate that the reasons that so much remain to be done is the result of having learned so much in this field. These advances as well as multifaceted approaches to the study of iron deficiency have the potential to serve as a model for the study of other nutrition problems.

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CLOSING REMARKS

George Hill
Director of Biomedical Sciences and
Associate Dean, School of Graduate Studies

This has been an excellent conference. We also work on parasites. Parasites in Africa and in South America (including schistosomiasis, malaria) and I hope they weren't maligned at this meeting. I certainly enjoyed the link between the types of research that are being conducted in the nutrition area and tropical diseases. In fact, as we try to expand the Tropical Disease Center that we have here, the impact of nutrition on parasitic infections is one area that we certainly would want to move into. I would certainly like to thank the speakers, and particularly those of you who have come from long distances. I know myself, when one has to go to a long meeting and then spend two to three days, it's really an imposition in terms of what's going on at home. You are always wondering what's going on, or not going on at home. We appreciate the time that you have been so eager and happy to spend with us.

I would like to thank Dr. Grossman and General Foods for their continued financial support and also the enthusiasm that they have for the center. It certainly would be remiss if I did not thank Dr. Enwonwu and his staff for again organizing an excellent conference here at Meharry.

The last point I'd like to make, deals with something that I have been reading lately. It concerns the goals for the nation by the year 2000 as defined by the Public Health Service. I'd like to read them. They are:

1) Reduced infant mortality to no more than seven deaths per thousand live births; 2) Increased life expectancy to at least 78 years;
3) Reduced disability caused by chronic conditions to a prevalence of no more than 6% of all people; 4) Increased years of healthy life to at least 65 years,
5) Decreased disparity in life expectancy between white and minority populations to no more than 4 years. I guess the thing that is flowing through all of these is the role of nutrition. And I certainly hope that as we all go back to our different institutions and centers, we remember these goals.

Again, for Dr. Jones, who's on his way to the AAMC meeting, Dr. Satcher, and those here at Meharry, we thank you all very much.

MEHARRY MEDICAL COLLEGE

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David Satcher, M.D., Ph. D., President, Meharry Medical College
Fred Jones, Ph.D., Vice President for Academic Affairs and Dean of Graduate Studies
Cyril O. Enwonwu, Sc.D., Ph.D., M.D.S., Director, Center for Nutrition

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